PATENT
Atty. Docket No.: 10192.0022

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent No. 5,925,730)
)
Issued: July 20, 1999)
)
To: Graeme Semple, Guangcheng Jiang,)
Jean E. F. Rivier)
)
Assignee: Ferring BV)
)
For: GNRH ANTAGONISTS)

ATTN: MAIL STOP HATCH-WAXMAN PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

APPLICATION FOR EXTENSION OF PATENT
TERM UNDER 35 U.S.C. § 156

Applicant, Ferring BV, represents that it is the Assignee of the entire interest in and to United States Patent No. 5,925,730 granted to Graeme Semple, Guangcheng Jiang, and Jean E. F. Rivier on the 20th day of July 1999, for GNRH Antagonists by virtue of an assignment from the inventors to Ferring BV, recorded in the U.S. Patent and Trademark Office at Reel 08641, Frame 0411 on July 10, 1997 and Reel 012831, Frame 0001 on June 26, 2002. By the Power of Attorney enclosed herein (Attachment A), Applicant appoints the practitioners associated with Finnegan, Henderson, Farabow, Garrett & Dunner, LLP, Customer No. 22852, including Charles E. Van Horn, to represent the assignee with regard to this application for

extension of the term of U.S. Patent No. 5,925,730 and to transact all business in the U.S. Patent and Trademark Office in connection therewith.

Information Required Under 37 C.F.R. § 1.740

Applicant hereby submits this application for extension of the patent term under 35 U.S.C. § 156 by providing the following information required by the rules promulgated by the U.S. Patent and Trademark Office (37 C.F.R. § 1.740). For the convenience of the Patent and Trademark Office, the information contained in this application will be presented in a format which follows the requirements of Section 1.740 of Title 37 of the Code of Federal Regulations.

(1) The approved product degarelix (tradename under review) is a GnRH receptor antagonist indicated for treatment of patients with advanced prostate cancer. It is a sterile lyophilized powder for injection containing degarelix (as the acetate) and mannitol. Degarelix is a synthetic linear decapeptide amide containing seven unnatural amino acids, five of which are D-amino acids.

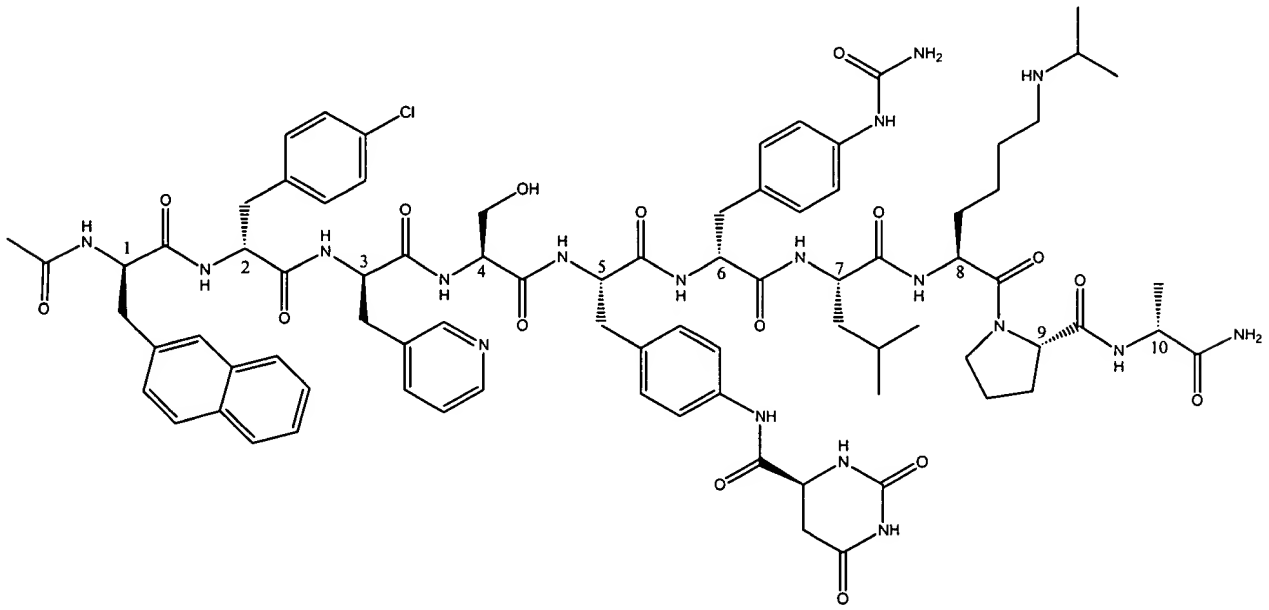
(2) The approved product was subject to regulatory review under § 505(b) of the Federal Food, Drug, and Cosmetic Act.

(3) The approved product received permission for commercial marketing or use under Section 505(b) of the Federal Food, Drug, and Cosmetic Act on December 24, 2008. A copy of the FDA letter approving the new drug application for degarelix for injection is attached (Attachment B).

(4) The active ingredient in the approved product is degarelix which, on information and belief, has not been approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act prior to approval by the Food and Drug Administration on December 24, 2008. A copy of a document describing the approved product is attached (Attachment C).

The chemical name of degarelix is D-Alaninamide, N-acetyl-3-(2-naphthalenyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridinyl)-D-alanyl-L-seryl-4-[[[(4S)-hexahydro-2,6-dioxo-4-pyrimidinyl]carbonyl]amino]-L phenylalanyl-4-[(aminocarbonyl)amino]-D-phenylalanyl-L leucyl-N6-(1-methylethyl)-L-lysyl-L-prolyl. It has an empirical formula of $C_{82}H_{103}N_{18}O_{16}Cl$ and a molecular weight of 1632.3 Da.

Degarelix has the following structural formula:



(5) This application for extension of patent term is being submitted within the permitted 60-day period pursuant to 35 U.S.C. § 156(d)(1) and 37 C.F.R. § 1.720(f),

said period will expire on February 21, 2009, if December 24, 2008, is day one (1) of the sixty (60) day period.

(6) The complete identification of the patent for which a term extension is being sought is as follows:

Inventors: Graeme Semple, Guangcheng Jiang, Jean E. F. Rivier

Patent No.: 5,925,730

Issue Date: July 20, 1999

Expiration Date: April 11, 2017

(7) A true copy of the patent is attached (Attachment D).

(8) No disclaimer has been filed and no reexamination certificate has been issued on this patent. A certificate of correction dated September 14, 2004, added Jean E. F. Rivier as an inventor. A copy of a record of maintenance fee payments under 35 U.S.C. § 41(b) is attached (Attachment E).

(9) U.S. Patent No. 5,925,730 claims the active ingredient (degarelix) in the approved product, a pharmaceutical composition comprising the active ingredient, and a method of using the active ingredient. The applicable patent claims are claims 1-3, 5-7, 10, 12, 14-16, and 18-20. The following description demonstrates the manner in which at least one claim reads on the active ingredient, a composition comprising the active ingredient, and a method of using the active ingredient.

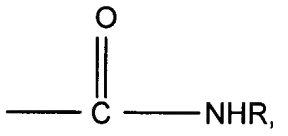
(a) Claim 1 reads as follows: A GnRH antagonist peptide having the formula:

X-D-2Nal-(A)D-Phe-D-3Pal-Ser-Xaa_5-Xaa_6-Leu-Xaa_8-Pro-Xaa_{10}

or pharmaceutically acceptable salt thereof wherein:

X is an acyl group having not more than 7 carbon atoms or Q,

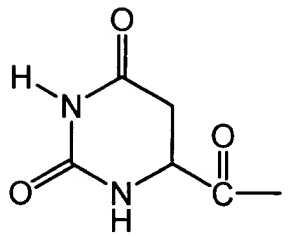
with Q being



and with R being H or lower alkyl;

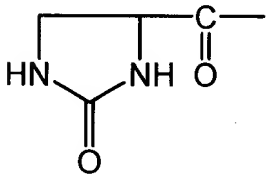
A is 4Cl, 4F, 4Br, $4NO_2$, $4CH_3$, $4OCH_3$, $3,4Cl_2$ or $C^{\alpha}Me4Cl$;

Xaa_5 is $4Aph(Q_1)$ or $4Amf(Q_1)$ with Q_1 being



(D— or L—Hor)

or



(D— or L—Imz)

Xaa_6 is D-$4Aph(Q_2)$, D-$4Amf(Q_2)$, D-Lys(Nic), D-Cit, D-Hci or D-3Pal, with Q_2 being For, Ac, 3-amino-1,2,4-triazole, Q or Q_1;

Xaa_8 is Lys(ipr), Arg, Har, $Arg(Et_2)$ or $Har(Et_2)$; and

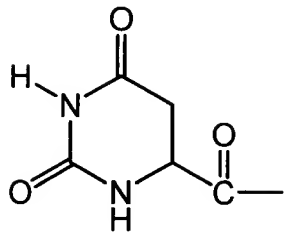
Xaa_{10} is D-Ala-NH_2, $NHCH_2CH_3$, Gly-NH_2, Ala-NH_2, AzaGly-NH_2, Agl-NH_2, D-Agl-NH_2, Agl(Me)-NH_2 or D-Agl(Me)-NH_2.

Claim 1 reads on the active ingredient, degarelix, when:

X is an acyl group having not more than 7 carbon atoms

A is 4 Cl

Xaa_5 is 4 Aph(Q_1) with Q_1 being



Xaa_6 is D-4Aph(Q_2) with Q_2 being Q where R is H

Xaa_8 is Lys (ipr)

Xaa_{10} is D-Ala-NH_2.

(b) Claim 19 reads as follows: A pharmaceutical composition for inhibiting the secretion of gonadotropins in mammals comprising, as an active ingredient, an effective amount of a GnRH antagonist according to claim 1 in association with a nontoxic diluent.

Claim 19 reads on a pharmaceutical composition comprising the active ingredient, degarelix, because it comprises an effective amount of a GnRH antagonist according to claim 1 which was shown above to read on degarelix.

(c) Claim 20 reads as follows: A method for inhibiting the secretion of gonadotropins in mammals comprising administering an amount of a pharmaceutical composition according to claim 19 which effects a substantial decrease in LH and FSH levels.

Claim 20 reads on a method of using the active ingredient, degarelix, because it recites administering the pharmaceutical composition of claim 19

which was shown above to read on a pharmaceutical composition containing degarelix.

While applicant has shown how representative claims read on the active ingredient degarelix, a composition containing it, and a process of using it, this should not be taken as an indication that these are the only claims in the patent that claim the product within the meaning of 35 U.S.C. §156(a).

(10) The relevant dates and information pursuant to 35 U.S.C. § 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

Investigational New Drug Application (IND 51,222) for degarelix was received by the FDA on July 11, 2001 and became effective on August 10, 2001.

We note that the brief description of activities contained in the next section (11) of this application for term extension indicates that the sponsor of IND 51,222 was notified of a Partial Clinical Hold by the FDA. We understand that this notice was first communicated to the sponsor in a telephone conference conducted on August 8, 2001, by FDA officials, and later in a letter dated August 15, 2008. As this clinical hold was only "partial," we do not regard that it would affect the effective date of IND 51,222 for purposes of determining the length of the regulatory review period under 35 U.S.C. § 156(g).

New Drug Application for degarelix (NDA 22-201) was submitted on February 28, 2008.

NDA 22-201 for degarelix was approved on December 24, 2008.

(11) A brief description of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to degarelix and the dates applicable to these significant activities are set forth in a chronology of events in Attachment F.

(12)(i) Applicant is of the opinion that U.S. Patent No. 5,925,730 is eligible for extension of the patent term under 35 U.S.C. § 156 because it satisfies all requirements for such extension as follows:

(a) 35 U.S.C. § 156(a) - U.S. Patent No. 5,925,730 claims a product (degarelix), a pharmaceutical composition comprising the product, and methods of using the product.

(b) 35 U.S.C. § 156(a)(1) - U.S. Patent No. 5,925,730 has not expired before submission of this application.

(c) 35 U.S.C. § 156(a)(2) - The term of U.S. Patent No. 5,925,730 has never been extended under 35 U.S.C. § 156(e)(1).

(d) 35 U.S.C. § 156(a)(3) - The application for patent term extension is submitted by the owner of record of the patent in accordance with the requirements of paragraphs (1) through (4) of 35 U.S.C. § 156(d) and the rules of the Patent and Trademark Office.

(e) 35 U.S.C. § 156(a)(4) - The product has been subjected to a regulatory review period before its commercial marketing or use.

(f) 35 U.S.C. § 156(a)(5)(A) - The commercial marketing or use of the product after the regulatory review period is the first permitted commercial marketing or use under § 505(b) of the Federal Food, Drug, and Cosmetic Act under which such regulatory review period occurred.

(g) 35 U.S.C. § 156(c)(4) - No other patent has been extended for the same regulatory review period for the product.

(12)(ii) Applicant respectfully submits that the length of the extension of patent term for U.S. Patent No. 5,925,720 is 1498 days pursuant to 35 U.S.C. § 156(c). The length of the extension was determined pursuant to 37 C.F.R. § 1.775 as follows:

(a) The regulatory review period under 35 U.S.C. § 156(g)(1)(B) began on August 10, 2001 and ended December 24, 2008, which is a total of 2695 days, which is the sum of (1) and (2) below:

(1) The period of review under 35 U.S.C. § 156(g)(1)(B)(i), the "Testing Period," began on August 10, 2001 and ended on February 28, 2008, which is 2394 days; and

(2) The period of review under 35 U.S.C. § 156(g)(1)(B)(ii), the "Approval Period," began on February 28, 2008, and ended on December 24, 2008, which is a total of 301 days.

(b) The regulatory review period upon which the period of extension is calculated is the entire regulatory review period as determined in subparagraph 12(ii)(a) above (2695) less:

(1) The number of days in the regulatory review period which were on or before the date on which the patent issued (July 20, 1999) which is zero (0) days; and

(2) The number of days during which applicant did not act with due diligence, which is zero (0) days; and

(3) One-half the number of days determined in subparagraph (12)(ii)(a)(1) above after the patent issued (one-half of 2394) which is 1197 days;

(c) The number of days as determined in subparagraph (12)(ii)(b) (1498 days) when added to the original term of the patent (April 11, 2017) would result in the date of May 18, 2021.

(d) Fourteen (14) years when added to the date of the NDA approval (December 24, 2008) would result in the date of December 24, 2022;

(e) The earlier date as determined in subparagraphs (12)(ii)(c) and (12)(ii)(d) is May 18, 2021;

(f) Since U.S. Patent No. 5,925,730 issued after September 24, 1984, the period of extension may not exceed five years from the original expiration date of April 11, 2017. Five years when added to the original expiration date of the patent would result in the date of April 11, 2022.

(g) The earlier date as determined by subparagraphs (12)(ii)(e) and (12)(ii)(f) is May 18, 2021.

(13) Applicant acknowledges a duty to disclose to the Director of the United States Patent and Trademark Office and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought.

(14) Please charge the prescribed fee for receiving and acting upon this application of $1,120.00 to Deposit Account No. 06-0916. The Director is authorized to charge any additional fees required by this application to Deposit Account No. 06-0916.

(15) All correspondence and inquiries may be directed to the undersigned, whose address, telephone number and fax number are as follows:

Customer No. 22852
Charles E. Van Horn
Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.
901 New York Avenue, N.W.
Washington, D.C. 20001-4413
Phone: 202-408-4000
Fax: 202-408-4400

(16) Enclosed is a certification that the application for extension of patent term under 35 U.S.C. § 156 including its attachments and supporting papers is being submitted as one original and two (2) copies thereof (Attachment G).

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

By: Charles E. Van Horn
Charles E. Van Horn
Reg. No. 40,266

Date: February 12, 2009

Attachments:
Power of Attorney (Attachment A)
Approval Letter (Attachment B)
Description of approved product (Attachment C)
U.S. Patent No. 5,925,730 (Attachment D)
Maintenance Fees Paid (Attachment E)
Chronology of Regulatory Review Period (Attachment F)
Certification of Copies of Application Papers (Attachment G)

ATTACHMENT A

In re U.S. Patent No. 5,925,730
Issued: July 20, 1999
To: Graeme Semple et al.
Assignee: Ferring BV
For: GNRH ANTAGONISTS
Application for Patent Term Extension
Customer No. 22852

PATENT
Attorney Docket No. 10192.0022

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent No. 5,925,730)
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Issued: July 20, 1999)
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To: Graeme Semple, Guangcheng Jiang, Jean E. F. Rivier)
)
Assignee: Ferring BV)
)
For: GNRH ANTAGONISTS)

ATTN: MAIL STOP HATCH-WAXMAN PTE **Attachment A**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

POWER OF ATTORNEY BY ASSIGNEE

The undersigned, a representative authorized to sign on behalf of the assignee owning all of the interest in this patent, verifies that Ferring BV is the assignee of the entire right, title, and interest in U.S. Patent No. 5,925,730 (the '730 patent) by virtue of an assignment from the inventors to Ferring BV, recorded in the U.S. Patent and Trademark Office at Reel 08641, Frame 0411 on July 10, 1997 and Reel 012831, Frame 0001 on June 26, 2002. To the best of the undersigned's knowledge and belief, title to the '730 patent is in the assignee.

The undersigned hereby grants power of attorney to the practitioners associated with Finnegan, Henderson, Farabow, Garrett & Dunner, LLP, Customer No. 22,852, including Charles E. Van Horn, both jointly and separately as attorneys with full power of substitution and revocation to prosecute the application for patent term extension of

the '730 patent and to transact all business in the Patent and Trademark Office connected therewith.

Please send all future correspondence concerning this application for patent term extension to Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P. at the following address:

Customer No. 22852
Finnegan, Henderson, Farabow,
Garrett & Dunner, L.L.P.
901 New York Avenue, N.W.
Washington, D.C. 20001-4413

Dated: February 9, 2009

By: _____

Name: Teddy Ercegovic
Senior Director, Group Patents

Title: _____

Assignee: FERRING BV

1777925

ATTACHMENT B

In re U.S. Patent No. 5,925,730
Issued: July 20, 1999
To: Graeme Semple et al.
Assignee: Ferring BV
For: GNRH ANTAGONISTS
Application for Patent Term Extension
Customer No. 22852

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Rockville, MD 20857

NDA 22-201

NDA APPROVAL

Ferring Pharmaceuticals, Inc.
Attention: Ronald Hargreaves, Ph.D.
4 Gatehall Drive, Third Floor
Parsippany, NJ 07054

Dear Dr. Hargreaves:

Please refer to your new drug application (NDA) dated February 14, 2008, received February 28, 2008, submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for degarelix for injection, 80 mg and 120 mg.

We acknowledge receipt of your amendments dated July 7 and 15, August 6, September 25, November 25, December 5, 8, 17, 18 (electronic), 19 (electronic), 22 (electronic), 23 (electronic), and 24 (2 electronic), 2008.

This new drug application provides for the use of degarelix for injection, 80 mg and 120 mg, for the treatment of patients with advanced prostate cancer.

We have completed our review of this application, as amended. It is approved, effective on the date of this letter, for use as recommended in the content of labeling [21 CFR 314.50(l)] in the enclosed agreed upon labeling.

This application was not referred to the Oncologic Drugs Advisory Committee for review because the key study used an established surrogate endpoint, and the results of the study did not raise significant issues with respect to the efficacy and safety of degarelix in the intended population.

PEDIATRIC RESEARCH EQUITY ACT (PREA)

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We are waiving the pediatric study requirement for this application.

POSTMARKETING REQUIREMENTS UNDER 505(o)

Title IX, Subtitle A, Section 901 of the Food and Drug Administration Amendments Act of 2007 (FDAAA) amends the Federal Food, Drug, and Cosmetic Act (FDCA) to authorize FDA to require holders of approved drug and biological product applications to conduct postmarketing studies and clinical trials for certain purposes, if FDA makes certain findings required by the statute (section 505(o)(3)(A), 21 U.S.C. 355(o)(3)(A)). This provision took effect on March 25, 2008.

We have determined that an analysis of spontaneous postmarketing adverse events reported under subsection 505(k)(1) of the FDCA will not be sufficient to identify an unexpected serious risk of hepatic toxicity or long-term safety specific to degarelix.

Furthermore, the new pharmacovigilance system that FDA is required to establish under section 505(k)(3) of the FDCA has not yet been established and is not sufficient to assess this serious risk.

Finally we have determined that only a clinical trial (rather than a nonclinical or observational study) will be sufficient to assess the serious risk of hepatic toxicity. In addition, long-term safety of degarelix administered at the recommended dosing schedule in the approval has not been established. Therefore, based on appropriate scientific data, FDA has determined that you are required, pursuant to section 505(o)(3) of the FDCA,

1. To complete the ongoing extension study FE200486 CS21A entitled "An Open-Label, Multi-Center, Extension Study, Evaluating the Long-Term Safety and Tolerability of Degarelix One Month Dosing Regimen in Patients with Prostate Cancer Requiring Androgen Ablation Therapy".

Protocol Submission:	January 2007
Trial Start Date:	March 2007
First Annual Report Submission:	March 2009
Second Annual Report Submission:	March 2010
Third Annual Report Submission:	March 2011
Final Report and Dataset Submission:	June 2012

Submit the protocol to your IND 51,222, with a cross-reference letter to this NDA 22-201. Submit all final report(s) to your NDA. Use the following designators to prominently label all submissions, including supplements, relating to this postmarketing requirement:

- **Required Postmarketing Protocol under 505(o)**
- **Required Postmarketing Final Report under 505(o)**
- **Required Postmarketing Correspondence under 505(o)**

Section 505(o)(3)(E)(ii) of the FDCA requires you to report periodically on the status of any study or clinical trial required under this section. This section also requires you to periodically report to FDA on the status of any study or clinical trial otherwise undertaken to investigate a safety issue.

Section 506B of the FDCA, as well as 21 CFR 314.81(b)(2)(vii) [or 21 CFR 601.70] requires you to report annually on the status of any postmarketing commitments or required studies or clinical trials.

FDA will consider the submission of your annual report under section 506B and 21 CFR 314.81(b)(2)(vii) [or 21 CFR 601.70] to satisfy the periodic reporting requirement under section 505(o)(3)(E)(ii) provided that you include the elements listed in 505(o) and 21 CFR 314.81(b)(2(vii) [or 21 CFR 601.70]. We remind you that to comply with 505(o), your annual report must also include a report on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Failure to submit an annual report for studies or clinical trials required under 505(o) on the date required will be considered a violation of FDCA section 505(o)(3)(E)(ii) and could result in enforcement action.

CONTENT OF LABELING

As soon as possible, but no later than 14 days from the date of this letter, please submit the content of labeling [21 CFR 314.50(l)] in structured product labeling (SPL) format as described at http://www.fda.gov/oc/datacouncil/spl.html that is identical to the enclosed labeling (text for the package insert and text for the patient package insert. Upon receipt, we will transmit that version to the National Library of Medicine for public dissemination. For administrative purposes, please designate this submission, "SPL for approved NDA 22-201."

CARTON AND IMMEDIATE CONTAINER LABELS

Submit final printed carton and container labels that are identical to the enclosed carton and immediate container labels as soon as they are available, but no more than 30 days after they are printed. Please submit these labels electronically according to the guidance for industry titled *Providing Regulatory Submissions in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (October 2005).* Alternatively, you may submit 12 paper copies, with 6 of the copies individually mounted on heavy-weight paper or similar material. For administrative purposes, designate this submission "**Final Printed Carton and Container Labels for approved NDA 22-201.**" Approval of this submission by FDA is not required before the labeling is used.

Marketing the product(s) with FPL that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

We note that you submitted the proposed proprietary name (b) (4) on November 25, 2008, and that it has not been reviewed in this review cycle. Upon receipt of this letter, we recommend that you re-submit your proposed proprietary name to the Agency for our review as a Prior Approval labeling supplement prior to its implementation.

PROMOTIONAL MATERIALS

You may request advisory comments on proposed introductory advertising and promotional labeling. To do so, submit, in triplicate, a cover letter requesting advisory comments, the proposed materials in draft or mock-up form with annotated references, and the package insert(s) to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Drug Marketing, Advertising, and Communications
5901-B Ammendale Road
Beltsville, MD 20705-1266

As required under 21 CFR 314.81(b)(3)(i), you must submit final promotional materials, and the package insert(s), at the time of initial dissemination or publication, accompanied by a Form FDA 2253. For instruction on completing the Form FDA 2253, see page 2 of the Form. For more information about submission of promotional materials to the Division of Drug Marketing, Advertising, and Communications (DDMAC), see www.fda.gov/cder/ddmac.

Please submit one market package of the drug product when it is available.

METHODS VALIDATION

We have not completed validation of the regulatory methods. However, we expect your continued cooperation to resolve any problems that may be identified.

LETTERS TO HEALTH CARE PROFESSIONALS

If you issue a letter communicating important safety related information about this drug product (i.e., a "Dear Health Care Professional" letter), we request that you submit an electronic copy of the letter to both this NDA and to the following address:

MedWatch
Food and Drug Administration
Suite 12B05
5600 Fishers Lane
Rockville, MD 20857

REPORTING REQUIREMENTS

We remind you that you must comply with reporting requirements for an approved NDA (21 CFR 314.80 and 314.81).

MEDWATCH-TO-MANUFACTURER PROGRAM

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at www.fda.gov/medwatch/report/mmp.htm.

If you have any questions, call Carl Huntley, Regulatory Project Manager, at (301) 796-1372.

Sincerely,

{See appended electronic signature page}

Richard Pazdur, M.D.
Director, Office of Oncology Drug Products
Office of New Drugs
Center for Drug Evaluation and Research
Food and Drug Administration

Enclosure

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Robert Justice
12/24/2008 04:40:09 PM
signing for Richard Pazdur, M.D.

ATTACHMENT C

In re U.S. Patent No. 5,925,730
Issued: July 20, 1999
To: Graeme Semple et al.
Assignee: Ferring BV
For: GNRH ANTAGONISTS
Application for Patent Term Extension
Customer No. 22852

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use TRADENAME safely and effectively. See full prescribing information for TRADENAME.

TRADENAME® (degarelix for injection) for subcutaneous administration
Initial U.S. Approval: 2008

----------INDICATIONS AND USAGE----------

TRADENAME is a GnRH receptor antagonist indicated for treatment of patients with advanced prostate cancer. (1)

----------DOSAGE AND ADMINISTRATION----------

- TRADENAME is for subcutaneous administration only and is not to be administered intravenously.
- Treatment is started with a dose of 240 mg given as two injections of 120 mg each.
- The starting dose is followed by maintenance doses of 80 mg administered as a single injection every 28 days. (2)

----------DOSAGE FORMS AND STRENGTHS----------

- TRADENAME (degarelix for injection) 120 mg per vial
- TRADENAME (degarelix for injection) 80 mg per vial

----------CONTRAINDICATIONS----------

Degarelix is contraindicated in:

- Patients with previous hypersensitivity reactions to TRADENAME. (4)
- Pregnancy Category X. Fetal harm can occur when administered to pregnant women. (4)

----------WARNINGS AND PRECAUTIONS----------

- Long-term androgen deprivation therapy prolongs the QT interval. Consider risks and benefits. (5.2)

----------ADVERSE REACTIONS----------

The most commonly observed adverse reactions (≥10%) during TRADENAME therapy included injection site reactions (e.g., pain, erythema, swelling or induration), hot flashes, increased weight, and increases in serum levels of transaminases and gamma-glutamyltransferase (GGT). (6)

To report SUSPECTED ADVERSE REACTIONS, contact Ferring at 1-888-FERRING (1-888-337-7464) or FDA at 1-800-FDA-1088 or *www.fda.gov/medwatch*.

----------DRUG INTERACTIONS----------

Clinically significant CYP450 pharmacokinetic drug-drug interactions are unlikely. (7)

----------USE IN SPECIFIC POPULATIONS----------

There is no need to adjust the dose for the elderly or in patients with mild or moderate liver or kidney function impairment. Patients with severe liver or kidney dysfunction have not been studied and caution is therefore warranted. (8)

See 17 for PATIENT COUNSELING INFORMATION (and FDA approved patient labeling)

Revised:12/2008

FULL PRESCRIBING INFORMATION: CONTENTS*

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FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

TRADENAME is a GnRH receptor antagonist indicated for treatment of patients with advanced prostate cancer.

2 DOSAGE AND ADMINISTRATION

TRADENAME is for subcutaneous administration only and is not to be administered intravenously.

Dosing information:

Starting dose	**Maintenance dose – Administration every 28 days**
240 mg given as two subcutaneous injections of 120 mg at a concentration of 40 mg/mL	80 mg given as one subcutaneous injection at a concentration of 20 mg/mL

The first maintenance dose should be given 28 days after the starting dose.

TRADENAME is administered as a subcutaneous injection in the abdominal region. As with other drugs administered by subcutaneous injection, the injection site should vary periodically. Injections should be given in areas of the abdomen that will not be exposed to pressure, e.g. not close to waistband or belt nor close to the ribs.

TRADENAME is supplied as a powder to be reconstituted with Sterile Water for Injection, USP.
The reconstitution procedure needs to be carefully followed. Administration of other concentrations is not recommended. See Instructions for Proper Use below.

Instructions for Proper Use

NOTE:

- Gloves should be worn during preparation and administration.
- Reconstituted drug must be administered within one hour after addition of Sterile Water for Injection, USP (WFI).
- Keep the vial vertical at all times
- Do not shake the vials
- Follow aseptic technique

TRADENAME 120 mg
The Treatment Initiation pack contains 2 vials of TRADENAME 120 mg that must be prepared for 2 subcutaneous injections. Hence, the instructions here below need to be repeated a second time.

Prepare TRADENAME 120 mg for reconstitution by gathering the following:

- 6 mL of Sterile Water for Injection, USP (WFI); Do not use Bacteriostatic Water for Injection.
- 2 reconstitution needles – 21G / 2 inch
- 2 administration needles for subcutaneous injection – 27 G / 1-1/4 inch
- 2 injection syringes (5 mL)

1. Draw up 3 mL WFI with a reconstitution needle (21G / 2 in).

2. Inject the WFI slowly into the TRADENAME 120 mg vial. To keep the product and syringe sterile, do not remove the syringe and the needle.

3. Keeping the vial in an upright position, swirl it very gently until the liquid looks clear and without undissolved powder or particles. If the powder adheres to the vial over the liquid surface, the vial can be tilted slightly to dissolve powder. Avoid shaking to prevent foam formation. A ring of small air bubbles on the surface of the liquid is acceptable. The reconstitution procedure may take up to 15 minutes.

4. Tilt the vial slightly and keep the needle in the lowest part of the vial. Withdraw 3 mL of TRADENAME 120 mg without turning the vial upside down.

5. Exchange the reconstitution needle with the administration needle for deep subcutaneous injection (27G / 1-1/4 in). Remove any air bubbles.

6. Inject 3 mL of TRADENAME 120 mg subcutaneously immediately after reconstitution.
   - Grasp the skin of the abdomen, elevate the subcutaneous tissue. Insert the needle deeply at an angle of not less than 45 degrees.
   - Gently pull back the plunger to check if blood is aspirated. If blood appears in the syringe, the reconstituted product can no longer be used. Discontinue the procedure and discard the syringe and the needle (reconstitute a new dose for the patient).

7. Repeat reconstitution procedure for the second dose. Choose a different injection site and inject 3 mL.

TRADENAME 80 mg
The Treatment Maintenance pack contains 1 vial of TRADENAME 80 mg that must be prepared for subcutaneous injection.

Prepare TRADENAME 80 mg for reconstitution by gathering the following:
- 4.2 mL of Sterile Water for Injection, USP (WFI); Do not use Bacteriostatic Water for Injection
- 1 reconstitution needle – 21G / 2 inch
- 1 administration needle for sc injection – 27 G / 1-1/4 inch
- 1 injection syringe (5 mL)

1. Draw up 4.2 mL WFI with the reconstitution needle (21G / 2 in).

2. Inject the WFI slowly into the TRADENAME 80 mg vial. To keep the product and syringe sterile, do not remove the syringe and the needle.

3. Keeping the vial in an upright position, swirl it very gently until the liquid looks clear and without undissolved powder or particles. If the powder adheres to the vial over the liquid surface, the vial can be tilted slightly to dissolve powder. Avoid shaking to prevent foam formation. A ring of small air bubbles on the surface of the liquid is acceptable. The reconstitution procedure may take up to 15 minutes.

4. Tilt the vial slightly and keep the needle in the lowest part of the vial. Withdraw 4 mL of TRADENAME 80 mg without turning the vial upside down.

5. Exchange the reconstitution needle with the administration needle for deep subcutaneous injection (27G / 1-1/4 in). Remove any air bubbles.

6. Inject 4 mL of TRADENAME 80 mg subcutaneously immediately after reconstitution.

- Grasp the skin of the abdomen, elevate the subcutaneous tissue. Insert the needle deeply at an angle of not less than 45 degrees.
- Gently pull back the plunger to check if blood is aspirated. If blood appears in the syringe, the reconstituted product can no longer be used. Discontinue the procedure and discard the syringe and the needle (reconstitute a new dose for the patient).

3 DOSAGE FORMS AND STRENGTHS

Starting dose

Powder for injection 120 mg:

One vial of TRADENAME 120 mg contains 120 mg degarelix. Each vial is to be reconstituted with 3 mL of Sterile Water for Injection. 3 mL is withdrawn to deliver 120 mg degarelix at a concentration of 40 mg/mL.

One starting dose comprises 240 mg given as two 3 mL injections of 120 mg each.

Maintenance dose

Powder for injection 80 mg:

One vial of TRADENAME 80 mg contains to 80 mg degarelix. Each vial is to be reconstituted with 4.2 mL of Sterile Water for Injection. 4 mL is withdrawn to deliver 80 mg degarelix at a concentration of 20 mg/mL.

One maintenance dose comprises 80 mg given as one 4 mL injection.

4 CONTRAINDICATIONS

TRADENAME is contraindicated in patients with known hypersensitivity to degarelix or to any of the product components.

Degarelix is contraindicated in women who are or may become pregnant. Degarelix can cause fetal harm when administered to a pregnant woman. Degarelix given to rabbits during organogenesis at doses that were 0.02% of the clinical loading dose (240 mg) on a mg/m^2 basis caused embryo/fetal lethality and abortion. When degarelix was given to female rats during organogenesis, at doses that were just 0.036% of the clinical loading dose on a mg/m^2 basis, there was an increase post implantation loss and a decrease in the number of live fetuses. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

5 WARNINGS AND PRECAUTIONS

5.1 Use in Pregnancy

Pregnancy Category X

Women who are or may become pregnant should not take TRADENAME. *[see Contraindications (4) and Use in Specific Populations (8.1)]*

5.2 Effect on QT/QTc Interval

Long-term androgen deprivation therapy prolongs the QT interval. Physicians should consider whether the benefits of androgen deprivation therapy outweigh the potential risks in patients with congenital long QT syndrome, electrolyte abnormalities, or congestive heart failure and in patients taking Class IA (e.g. quinidine, procainamide) or Class III (e.g. amiodarone, sotalol) antiarrhythmic medications.

In the randomized, active-controlled trial comparing TRADENAME to leuprolide, periodic electrocardiograms were performed. Seven patients, three (<1%) in the pooled degarelix group and four (2%) patients in the leuprolide 7.5 mg group, had a QTcF ≥ 500 ms. From baseline to end of study the median change for TRADENAME was 12.3 msec and for leuprolide was 16.7 msec.

5.3 Laboratory Testing

Therapy with TRADENAME results in suppression of the pituitary gonadal system. Results of diagnostic tests of the pituitary gonadotropic and gonadal functions conducted during and after TRADENAME may be affected.

The therapeutic effect of TRADENAME should be monitored by measuring serum concentrations of prostate-specific antigen (PSA) periodically. If PSA increases, serum concentrations of testosterone should be measured.

6 ADVERSE REACTIONS

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

A total of 1325 patients with prostate cancer received TRADENAME either as a monthly treatment (60-160 mg) or as a single dose (up to 320 mg). A total of 1032 patients (78%) were treated for at least 6 months and 853 patients (64%) were treated for one year or more. The most commonly observed adverse reactions during TRADENAME therapy included injection site reactions (e.g. pain, erythema, swelling or induration), hot flashes, increased weight, fatigue, and increases in serum levels of transaminases and gamma-glutamyltransferase (GGT). The majority of the adverse reactions were Grade 1 or 2, with Grade 3/4 adverse reaction incidences of 1% or less.

TRADENAME was studied in an active-controlled trial (N = 610) in which patients with prostate cancer were randomized to receive TRADENAME (subcutaneous) or leuprolide (intramuscular) monthly for 12 months. Adverse reactions reported in 5% of patients or more are shown in Table 1.

Table 1. Adverse Reactions Reported in ≥ 5% of Patients in an Active Controlled Study

	TRADENAME 240/160 mg (subcutaneous) N = 202	TRADENAME 240/80 mg (subcutaneous) N = 207	leuprolide 7.5 mg (intramuscular) N = 201
	%	%	%
Percentage of subjects with adverse events	83	79	78
Body as a whole			
Injection site adverse events	44	35	<1
Weight increase	11	9	12
Fatigue	6	3	6
Chills	4	5	0
Cardiovascular system			
Hot flash	26	26	21
Hypertension	7	6	4
Musculoskeletal system			
Back pain	6	6	8
Arthralgia	4	5	9
Urogenital system			
Urinary tract infection	2	5	9
Digestive system			
Increases in Transaminases and GGT	10	10	5
Constipation	3	5	5

The most frequently reported adverse reactions at the injection sites were pain (28%), erythema (17%), swelling (6%), induration (4%) and nodule (3%). These adverse reactions were mostly transient, of mild to moderate intensity, occurred primarily with the starting dose and led to few discontinuations (<1%). Grade 3 injection site reactions occurred in 2% or less of patients receiving degarelix.

Hepatic laboratory abnormalities were primarily Grade 1 or 2 and were generally reversible. Grade 3 hepatic laboratory abnormalities occurred in less than 1% of patients.

In 1-5% of patients the following adverse reactions, not already listed, were considered related to TRADENAME by the investigator:

Body as a whole: Asthenia, fever, night sweats; *Digestive system:* Nausea; *Nervous system:* Dizziness, headache, insomnia.

The following adverse reactions, not already listed, were reported to be drug-related by the investigator in ≥1% of patients: erectile dysfunction, gynecomastia, hyperhidrosis, testicular atrophy, and diarrhea.

Changes in bone density:
Decreased bone density has been reported in the medical literature in men who have had orchiectomy or who have been treated with a GnRH agonist. It can be anticipated that long periods of medical castration in men will result in decreased bone density.

Anti-degarelix antibody development has been observed in 10% of patients after treatment with TRADENAME

for 1 year. There is no indication that the efficacy or safety of TRADENAME treatment is affected by antibody formation.

7 DRUG INTERACTIONS

No drug-drug interaction studies were conducted.

Degarelix is not a substrate for the human CYP450 system. Degarelix is not an inducer or inhibitor of the CYP450 system *in vitro*. Therefore, clinically significant CYP450 pharmacokinetic drug-drug interactions are unlikely.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Category X *[see Contraindications (4) and Warnings and Precautions (5.1)]*

Women who are or may become pregnant should not take TRADENAME.
When degarelix was given to rabbits during early organogenesis at doses of 0.002 mg/kg/day (about 0.02% of the clinical loading dose on a mg/m^2 basis), there was an increase in early post-implantation loss. Degarelix given to rabbits during mid and late organogenesis at doses of 0.006 mg/kg/day (about 0.05% of the clinical loading dose on a mg/m^2 basis) caused embryo/fetal lethality and abortion. When degarelix was given to female rats during early organogenesis, at doses of 0.0045 mg/kg/day (about 0.036% of the clinical loading dose on a mg/m^2 basis), there was an increase in early post-implantation loss. When degarelix was given to female rats during mid and late organogenesis, at doses of 0.045 mg/kg/day (about 0.36% of the clinical loading dose on a mg/m^2 basis), there was an increase in the number of minor skeletal abnormalities and variants.

8.3 Nursing Mothers

TRADENAME is not indicated for use in women and is contraindicated in women who are or who may become pregnant. It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from degarelix, a decision should be made whether to discontinue nursing or discontinue the drug taking into account the importance of the drug to the mother.

8.4 Pediatric Use

Safety and effectiveness in pediatric patients have not been established.

8.5 Geriatric Use

Of the total number of subjects in clinical studies of TRADENAME, 82% were age 65 and over, while 42% were age 75 and over. No overall differences in safety or effectiveness were observed between these subjects and younger subjects, but greater sensitivity of some older individuals cannot be ruled out.

8.6 Renal Impairment

No pharmacokinetic studies in renally impaired patients have been conducted. At least 20-30% of a given dose of degarelix is excreted unchanged in the urine.

A population pharmacokinetic analysis of data from the randomized study demonstrated that there is no significant effect of mild renal impairment [creatinine clearance (CrCL) 50-80 mL/min] on either the degarelix concentration or testosterone concentration. Data on patients with moderate or severe renal impairment is limited and therefore degarelix should be used with caution in patients with CrCL < 50 mL/min.

8.7 Hepatic Impairment

Patients with hepatic impairment were excluded from the randomized trial.

A single dose of 1 mg degarelix administered as an intravenous infusion over 1 hour was studied in 16 non-prostate cancer patients with either mild (Child Pugh A) or moderate (Child Pugh B) hepatic impairment. Compared to non-prostate cancer patients with normal liver function, the exposure of degarelix decreased by 10% and 18% in patients with mild and moderate hepatic impairment, respectively. Therefore, dose adjustment is not necessary in patients with mild or moderate hepatic impairment. However, since hepatic impairment can lower degarelix exposure, it is recommended that in patients with hepatic impairment testosterone concentrations should be monitored on a monthly basis until medical castration is achieved. Once medical castration is achieved, an every-other-month testosterone monitoring approach could be considered.

Patients with severe hepatic dysfunction have not been studied and caution is therefore warranted in this group.

10 OVERDOSAGE

There have been no reports of overdose with TRADENAME. In the case of overdose, however, discontinue TRADENAME, treat the patient symptomatically, and institute supportive measures.

As with all prescription drugs, this medicine should be kept out of the reach of children.

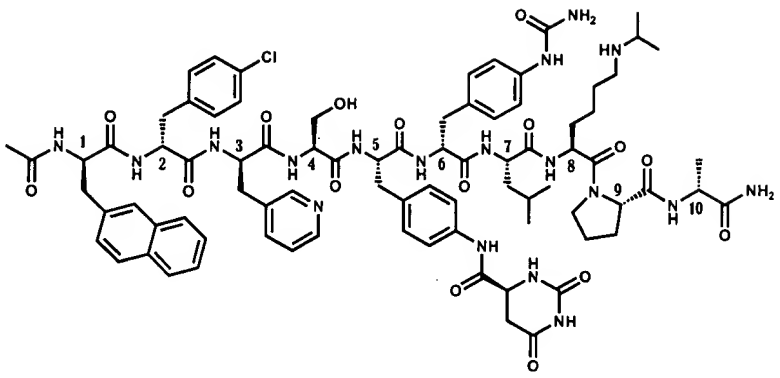
SEE TRADENAME PATIENT COUNSELING INFORMATION

11 DESCRIPTION

TRADENAME is a sterile lyophilized powder for injection containing degarelix (as the acetate) and mannitol. Degarelix is a synthetic linear decapeptide amide containing seven unnatural amino acids, five of which are D-amino acids. The acetate salt of degarelix is a white to off-white amorphous powder of low density as obtained after lyophilization.

The chemical name of degarelix is D-Alaninamide, N-acetyl-3-(2-naphthalenyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridinyl)-D-alanyl-L-seryl-4-[[[(4S)-hexahydro-2,6-dioxo-4-pyrimidinyl]carbonyl]amino]-L phenylalanyl-4-[(aminocarbonyl)amino]-D-phenylalanyl-L leucyl-N6–(1-methylethyl)-L-lysyl-L-prolyl. It has an empirical formula of $C_{82}H_{103}N_{18}O_{16}Cl$ and a molecular weight of 1632.3 Da.

Degarelix has the following structural formula:



TRADENAME delivers degarelix acetate, equivalent to 120 mg of degarelix for the starting dose, and 80 mg of degarelix for the maintenance dose. The 80 mg vial contains 200 mg mannitol and the 120 mg vial contains 150 mg mannitol.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

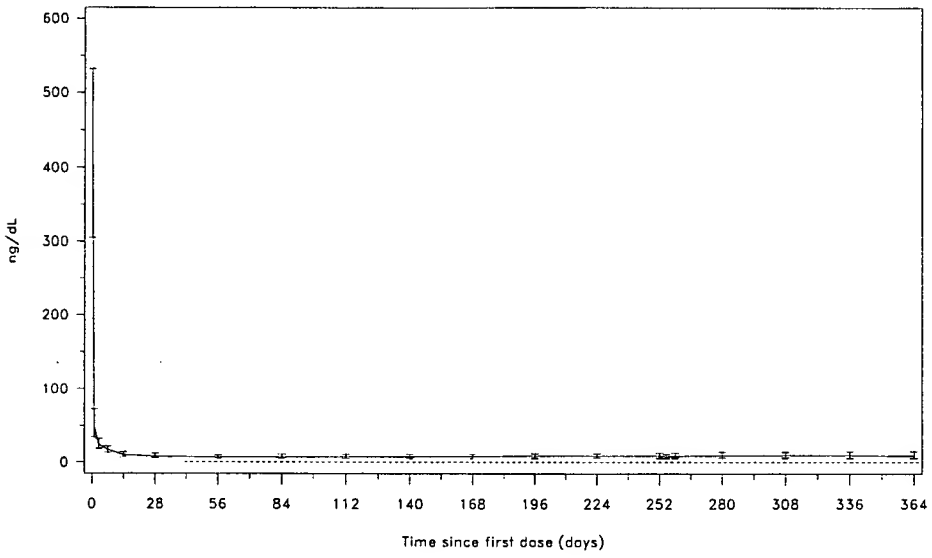
Degarelix is a GnRH receptor antagonist. It binds reversibly to the pituitary GnRH receptors, thereby reducing the release of gonadotropins and consequently testosterone.

12.2 Pharmacodynamics

A single dose of 240 mg TRADENAME causes a decrease in the plasma concentrations of luteinizing hormone (LH) and follicle stimulating hormone (FSH), and subsequently testosterone.

TRADENAME is effective in achieving and maintaining testosterone suppression below the castration level of 50 ng/dL.

Figure 1: Plasma Testosterone Levels from Day 0 to 364 for Degarelix 240 mg/80 mg (Median with Interquartile Ranges)



12.3 Pharmacokinetics

Absorption

TRADENAME forms a depot upon subcutaneous administration, from which degarelix is released to the circulation. Following administration of TRADENAME 240 mg at a product concentration of 40 mg/mL, the mean Cmax was 26.2 ng/mL (coefficient of variation, CV 83%) and the mean AUC was 1054 ng·day/mL (CV 35%). Typically Cmax occurred within 2 days after subcutaneous administration. In prostate cancer patients at a product concentration of 40 mg/mL, the pharmacokinetics of degarelix were linear over a dose range of 120 to 240 mg. The pharmacokinetic behavior of the drug is strongly influenced by its concentration in the injection solution.

Distribution

The distribution volume of degarelix after intravenous (> 1 L/kg) or subcutaneous administration (> 1000L) indicates that degarelix is distributed throughout total body water. *In vitro* plasma protein binding of degarelix is estimated to be approximately 90%.

Metabolism

Degarelix is subject to peptide hydrolysis during the passage of the hepato-biliary system and is mainly excreted as peptide fragments in the feces. No quantitatively significant metabolites were detected in plasma samples after subcutaneous administration. *In vitro* studies have shown that degarelix is not a substrate, inducer or inhibitor of the CYP450 or p-glycoprotein transporter systems.

Excretion

Following subcutaneous administration of 240 mg TRADENAME at a concentration of 40 mg/mL to prostate cancer patients, degarelix is eliminated in a biphasic fashion, with a median terminal half-life of approximately 53 days. The long half-life after subcutaneous administration is a consequence of a very slow release of degarelix from the TRADENAME depot formed at the injection site(s). Approximately 20-30% of a given dose of degarelix was renally excreted, suggesting that approximately 70-80% is excreted via the hepato-biliary system in humans. Following subcutaneous administration of degarelix to prostate cancer patients the clearance is approximately 9 L/hr.

Effect of Age, Weight and Race

There was no effect of age, weight or race on the degarelix pharmacokinetic parameters or testosterone concentration.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Degarelix was administered subcutaneously to rats every 2 weeks for 2 years at doses of 2, 10 and 25 mg/kg (about 9, 45 and 120% of the recommended human loading dose on a mg/m^2 basis). Long term treatment with degarelix at 25 mg/kg caused an increase in the combined incidence of benign hemangiomas plus malignant hemangiosarcomas in females.

Degarelix was administered subcutaneously to mice every 2 weeks for 2 years at doses of 2, 10 and 50 mg/kg (about 5, 22 and 120% of the recommended human loading dose (240 mg) on a mg/m^2 basis). There was no statistically significant increase in tumor incidence associated with this treatment.

Degarelix did not cause genetic damage in standard *in vitro* assays (bacterial mutation, human lymphocyte chromosome aberration) nor in *in vivo* rodent bone marrow micronucleus tests.

Single degarelix doses of $\geq$ 1 mg/kg (about 5% of the clinical loading dose on a mg/m^2 basis) caused reversible infertility in male rats. Single doses of $\geq$ 0.1 mg/kg (about 0.5% of the clinical loading dose on a mg/m^2 basis) caused a decrease in fertility in female rats.

14 CLINICAL STUDIES

The safety and efficacy of TRADENAME were evaluated in an open-label, multi-center, randomized, parallel-group study in patients with prostate cancer A total of 620 patients were randomized to receive one of two TRADENAME dosing regimens or leuprolide for one year:

a. TRADENAME at a starting dose of 240 mg (40 mg/mL) followed by monthly doses of 160 mg (40 mg/mL) subcutaneously,
b. TRADENAME at a starting dose of 240 mg (40 mg/mL) followed by monthly doses of 80 mg (20 mg/mL) subcutaneously,
c. leuprolide 7.5 mg intramuscularly monthly.

Serum levels of testosterone were measured at screening, on days 0, 1, 3, 7, 14, and 28 in the first month, and then monthly until the end of the study.

The clinical trial population (n=610) across all treatment arms had an overall median age of approximately 73 (range 50 to 98). The ethnic/racial distribution was 84% white, 6% black and 10% others. Disease stage was distributed approximately as follows: 20% metastatic, 29% locally advanced (T3/T4 Nx M0 or N1 M0), 31% localized (T1 or T2 N0 M0) and 20% classified as other (including patients whose disease metastatic status could not be determined definitively - or patients with PSA relapse after primary curative therapy). In addition, the median testosterone baseline value across treatment arms was approximately 400 ng/dL.

The primary objective was to demonstrate that TRADENAME is effective with respect to achieving and maintaining testosterone suppression to castration levels (T ≤ 50 ng/dL), during 12 months treatment. The results are shown in Table 2.

Table 2: Medical Castration Rates (Testosterone ≤ 50 ng/dL) from Day 28 to Day 364

	TRADENAME 240/160 mg N=202	TRADENAME 240/80 mg N=207	leuprolide 7.5 mg N=201
No. of Responders	199	202	194
Castration Rate (95% CIs)*	98.3% (94.8; 99.4)	97.2% (93.5; 98.8%)	96.4% (92.5; 98.2%)

* Kaplan Meier estimates within group

Percentage changes in testosterone from baseline to day 28 (median with interquartile ranges) are shown in Figure 2 and the percentages of patients who attained the medical castration of testosterone ≤ 50 ng/dL are summarized in Table 3.

Figure 2: Percentage Change in Testosterone from Baseline by Treatment Group until Day 28 (Median with Interquartile Ranges)

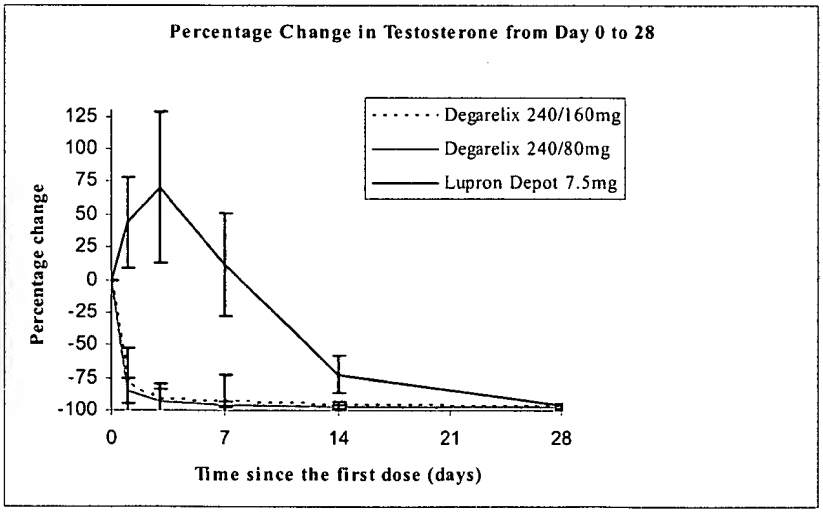


Table 3: Percentage of Patients Attaining Testosterone ≤ 50 ng/dL within the First 28 Days

	Degarelix (240/160 mg) N=202	Degarelix (240/80 mg) N=207	Leuprolide (7.5 mg) N=201
Day 1	44%	52%	0%
Day 3	96%	96%	0%
Day 7	99%	99%	1%
Day 14	99%	99%	18%
Day 28	99%	100%	100%

In the clinical trial, PSA levels were monitored as a secondary endpoint. PSA levels were lowered by 64% two weeks after administration of TRADENAME, 85% after one month, 95% after three months, and remained suppressed throughout the one year of treatment. These PSA results should be interpreted with caution because of the heterogeneity of the patient population studied. No evidence has shown that the rapidity of PSA decline is related to a clinical benefit.

15 REFERENCES

1. NIOSH Alert: Preventing occupational exposures to antineoplastic and other hazardous drugs in healthcare settings. 2004. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2004-165.
2. OSHA Technical Manual, TED 1-0.15A, Section VI: Chapter 2. Controlling Occupational Exposure to Hazardous Drugs. OSHA, 1999. http://www.osha.gov/dts/osta/otm/otm_vi/otm_vi_2.html
3. American Society of Health-System Pharmacists. ASHP guidelines on handling hazardous drugs. *Am J Health-Syst Pharm.* 2006; 63:1172-1193.
4. Polovich, M., White, J. M., & Kelleher, L.O. (eds.) 2005. Chemotherapy and biotherapy guidelines and recommendations for practice (2nd. ed.) Pittsburgh, PA: Oncology Nursing Society.

16 HOW SUPPLIED/STORAGE AND HANDLING

TRADENAME is available as:

- NDC 55566-8401-1, Starting dose – One carton contains:
  Two vials each with 120 mg powder for injection

- NDC 55566-8301-1, Maintenance dose – One carton contains:
  One vial with 80 mg powder for injection

Store at 25°C (77°F); excursions permitted to 15-30°C (59-86°F).

Caution should be exercised in handling and preparing the solution of TRADENAME. Several guidelines on proper handling and disposal of anticancer drugs have been published.[1-4] To minimize the risk of dermal exposure, always wear impervious gloves when handling TRADENAME. If TRADENAME solution contacts the skin, immediately wash the skin thoroughly with soap and water. If TRADENAME contacts mucous membranes, the membranes should be flushed immediately and thoroughly with water *[see Contraindications (4) and Nonclinical Toxicology (13.1)]*.

17 PATIENT COUNSELING INFORMATION

(See FDA-approved Patient Labeling 17.2)

17.1 Information

- Patients should be instructed to read the Patient Labeling carefully.
- Patients should be informed of the possible side effects of androgen deprivation therapy, including hot flashes, flushing of the skin, increased weight, decreased sex drive, and difficulties with erectile function. Possible side effects related to therapy with TRADENAME include redness, swelling, and itching at the injection site; these are usually mild, self limiting, and decrease within three days.

17.2 FDA-approved Patient Labeling

TRADENAME (phonetic spelling of TRADENAME)
(degarelix for injection)

Read this patient information leaflet before you start taking TRADENAME and each time you get a refill. There may be new information. This information does not take the place of talking to your healthcare provider about your medical condition or your treatment.

What is TRADENAME?

TRADENAME is a prescrpition medicine used in the treatment of advanced prostate cancer.

It is not known if TRADENAME is safe or effective in children.

Who should not use TRADENAME?

TRADENAME should not be given to:

- people who are allergic to any of the other ingredients in TRADENAME. See the end of this leaflet for a complete list of ingredients in TRADENAME
- women who are pregnant or may become pregnant

Talk to your healthcare provider before getting TRADENAME if you have any of these conditions.

What should I tell my healthcare provider before receiving TRADENAME?

Before receiving TRADENAME, tell your healthcare provider about all your medical conditions, including if you:

- have any heart problems
- have problems with balance of your body salts or electrolytes, such as sodium, potassium, calcium,and magnesium
- have kidney or liver problems
- are breast-feeding or plan to breast-feed. It is not known if TRADENAME passes into your breast milk. You and your healthcare provider should decide if you will take TRADENAME or breast feed. You should not do both without talking with your healthcare provider.

Tell your healthcare provider about all the medicines you take, including prescription and nonprescription medicines, vitamins, and herbal supplements. Especially tell your healthcare provider if you are taking or have taken any medicines for your heart.

Know the medicines you take. Keep a list of them and show it to your healthcare provider and pharmacist when you get a new medicine.

How should I receive TRADENAME?

You will receive an injection of TRADENAME from your healthcare provider.

- The injection site will always be in the abdominal area but will change within that area with the next doses of TRADENAME.
- The injected medicine gives you a continuous release of TRADENAME over one month.
- Two injections are given as a first dose and the following monthly doses are one injection.
- Make sure your injection site is free of any pressure from belts, waistbands or other types of clothing.
- Always set up an appointment for your next injection.
- If you miss a dose of TRADENAME, or if you think you forgot to get your monthly dose of TRADENAME, talk to your healthcare provider about how to get your next dose.

What are the possible side effects of TRADENAME?

The common side effects include:

- hot flashes
- injection site pain, redness, and swelling, especially with the first dose
- weight gain
- increase in some liver enzymes
- tiredness
- hypertension
- back and joint pain
- chills
- urinary tract infection
- decreased sex drive and trouble with erectile function (impotence)

These are not all the possible side effects. For more information, ask your healthcare provider or pharmacist.

Tell your healthcare provider if you have any side effect that bothers you or that does not go away.

Call your doctor for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

General information about the safe and effective use of TRADENAME.

Medicines are sometimes prescribed for conditions that are not mentioned in the patient leaflet. Do not use TRADENAME for a condition for which it was not prescribed. Do not give TRADENAME to other people, even if they have the same symptoms that you have. It may harm them.

This patient information leaflet summarizes the most important information about TRADENAME. If you would like more information, talk with your healthcare provider. You can ask your pharmacist or healthcare provider for information about TRADENAME that is written for health professionals.

For more information, go to www.TRADENAME.com or call 1-888-FERRING (1-888-337-7464)

What are the ingredients in TRADENAME?

Active ingredient: degarelix (as acetate)
Inactive ingredient: mannitol

Manufactured for: Ferring Pharmaceuticals Inc.

ATTACHMENT D

In re U.S. Patent No. 5,925,730
Issued: July 20, 1999
To: Graeme Semple et al.
Assignee: Ferring BV
For: GNRH ANTAGONISTS
Application for Patent Term Extension
Customer No. 22852

Attachment D

US005925730A

United States Patent [19]

Semple et al.

[11] **Patent Number: 5,925,730**

[45] **Date of Patent: Jul. 20, 1999**

[54] **GNRH ANTAGONISTS**

[75] Inventors: **Graeme Semple**, Hampshire, United Kingdom; **Guangcheng Jiang**, San Diego, Calif.

[73] Assignee: **Ferring BV**, Hoofddorp, Netherlands

[21] Appl. No.: **08/837,042**

[22] Filed: **Apr. 11, 1997**

[51] **Int. Cl.**[6] **C07K 7/00**

[52] **U.S. Cl.** **530/328**; 530/313; 514/15; 514/800; 930/110

[58] **Field of Search** 514/15, 800; 530/313, 530/328; 930/110

[56] **References Cited**

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Primary Examiner—Robert J. Hill, Jr.
Assistant Examiner—C. Delacroix-Muirheid
Attorney, Agent, or Firm—Fitch, Even, Tabin & Flannery

[57] **ABSTRACT**

Peptides are provided which have improved duration of GnRH antagonistic properties. These antagonists may be used to regulate fertility and to treat steroid-dependent tumors and for other short-term and long-term treatment indications. These antagonists have a derivative of aminoPhe or its equivalent in the 5- and/or 6-positions. This derivative contains a carbamoyl group or a heterocycle including a urea in its side chain. Particularly effective decapeptides, which continue to exhibit very substantial suppression of LH secretion at 96 hours following injection, have the formulae: Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph (hydroorotyl)-D-4Aph(acetyl)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2, and Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph (hydroorotyl)-D-4Amf(Q_2)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2, wherein Q_2 is Cbm or MeCbm.

21 Claims, No Drawings

GNRH ANTAGONISTS

This invention relates generally to peptides which are antagonists of human gonadotropin releasing hormone (GnRH) and which have advantageous physical, chemical and biological properties. More particularly, the present invention relates to decapeptides which inhibit the gonadal function and the release of the steroidal hormones progesterone and testosterone for periods of longer duration, and to methods of administering pharmaceutical compositions containing such decapeptides for such purpose and particularly to manage conditions resulting from the hypersecretion of gonadal steroids.

BACKGROUND OF THE INVENTION

Follicle stimulating hormone (FSH) and luteinizing hormone (LH), sometimes referred to as gonadotropins or gonadotropic hormones, are released by the pituitary gland which is attached by a stalk to the region in the base of the brain known as the hypothalamus. These hormones, in combination, regulate the functioning of the gonads to produce testosterone in the testes and progesterone and estrogen in the ovaries, and they also have other biological functions.

Hormone release by the anterior lobe of the pituitary gland usually requires prior release of hormones produced by the hypothalamus. A hypothalamic hormone which triggers the release of the gonadotropic hormones, particularly LH, is generally now referred to as GnRH. GnRH was isolated and characterized as a decapeptide some 25 years ago. Shortly thereafter, it was found that analogs of GnRH having a D-isomer instead of Gly in the 6-position have greater binding affinity/strength to the receptor and greater biological potency than the native hormone; one example is [D-Ala^6]-GnRH (U.S. Pat. No. 4,072,668) having the following formula: pGlu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-Gly-NH_2.

The formula for the GnRH analog represented above is in accordance with conventional representation of peptides where the amino terminus appears to the left and the carboxyl terminus to the right. The position of each amino acid residue is identified by numbering the amino acid residues from left to right. In the case of GnRH, the hydroxyl portion of the carboxyl group of glycine at the C-terminus has been replaced with an amino group(NH_2) i.e. the C-terminus is amidated. The abbreviations for the individual amino acid residues above are conventional. Except for glycine, the amino acids and other modifiers attached thereto set forth hereinafter should be understood to be of the L-configuration unless noted otherwise to be the D-isomer.

The administration of GnRH analogs that are antagonistic to the normal function of GnRH has been used to suppress secretion of gonadotropins generally in mammals and to suppress or delay ovulation. Because GnRH antagonists are capable of immediate inhibition of pituitary gonadotropin secretion by competing with the stimulatory effect of endogenous GnRH, such analogs of GnRH are being investigated for their potential use as suppressives, as contraceptives and for regulating conception periods and for the control of the timing of ovulation for in vitro fertilization. For example, GnRH antagonists may be used for the treatment of precocious puberty and endometriosis and other such conditions which result from hypersecretion of gonadotropins, and they are also useful for regulating the secretion of gonadotropins in male mammals, where they can be employed to arrest spermatogenesis, e.g. as male contraceptives, for treatment

of male sex offenders, and for treatment of prostatic hypertrophy. GnRH antagonists are also used to treat steroid-dependent tumors, such as prostatic and mammary tumors. In the female, they can also be used to treat hirsutism. GnRH antagonists offer advantages over the currently available, lengthy administration regimen of GnRH agonists, such as the absence of an initial gonadotropin stimulation (flare) and the dose proportional efficacy.

The development of these compounds has been hampered by histamine-release inducing properties, i.e. cause histamine to be released from mast cells which cells are found in the skin, the gingiva and other locations throughout the body. As a result, inflammation is caused, at times resulting in edema of the face and elsewhere on the skin. Certain GnRH antagonists that are effective in preventing ovulation have the undesirable adverse side effect of stimulating histamine release; thus, the design of GnRH analogs has generally been directed to providing peptides that retain the biological efficacy but do not exhibit such undesirable histamine release, see J. Rivier et al., *J. Med. Chem.,* 29, 1846–1851 (1986). The occurrence of depot formation after injection due to "gelling" results in release from the injection site that may be difficult to control, and improvements in solubility of these peptides have been sought to avoid such gelling.

The aim of GnRH antagonists is generally to suppress endogenous gonadotropins and/or sex steroids, and such suppression may be required for either short periods of time (e.g. during infertility treatment) or for long periods (e.g. during the treatment of endocrine cancers). Depending on the specific indication, short-term treatment varies from 1 day to about 6 weeks, whereas long-term treatment may last from several months to many years. A subdivision in short- and long-term treatment has a practical background. Presently available pharmaceutical formulations of GnRH antagonists permit daily subcutaneous (sc) administration only; therefore, long-acting, sustained-release formulations are required if one is to effect long-term treatment, with such formulations being only in the early stages of pharmaceutical development.

Short-term GnRH antagonist treatment is anticipated to be effective in the following situations:

(1) diagnostic;

(2) prevention of luteinizing hormone (LH) surges in controlled ovarian hyperstimulation (COH) for assisted reproductive techniques;

(3) suppression of increased LH levels during induction of ovulation in polycystic ovarian disease (PCOD) to decrease the incidence of spontaneous abortion;

(4) premenstrual syndrome (PMS);

(5) treatment of threatening ovarian hyperstimulation syndrome (OHSS);

(6) preparation for surgery of leiomyoma;

(7) functional menometrorrhagia;

(8) male contraception by (initiating the) suppression of gonadotropins;

(9) protection of the gonads during cytostatic treatment for cancer; and

(10) interval treatment of endometrial cancer between diagnosis and surgery.

Long-term GnRH-antagonist treatment (several months to many years) is expected to be effective treatment in the following indications;

(1) prostate cancer;

(2) breast cancer;

(3) endometrial cancer;

(4) ovarian cancer;

(5) benign prostatic hypertrophia;

(6) precocious puberty;

(7) endometriosis;

(8) hyperandrogenism; and

(9) promotion of hair growth.

Presently, the long period of treatment for these indications has been considered to require a sustained release GnRH antagonist depot preparation because daily sc injections are generally considered to be unacceptable. Linkage of GnRH analogs to cytotoxic radicals may increase the efficacy of cancer treatment using these compounds with a concomitant decrease of general toxicity.

The search for improved GnRH antagonists has resulted in the making of Antide, i.e. [Ac-D-2Nal^1, D-4ClPhe^2, D-3Pal^3, Lys(Nic)^5, D-Lys(Nic)^6, ILys^8, D-Ala^{10}]-GnRH; and Cetrorelix, i.e. [Ac-D-2Nal^1, D-4ClPhe^2, D-3Pal^3, D-Cit^6, D-Ala^{10}]-GnRH. U.S. Pat. No. 5,516,887 describes GnRH antagonists which are said to be more effective than Antide in suppressing plasma testosterone, e.g. [Ac-D-2Nal^1, D-4ClPhe^2, D-3Pal^3, D-N$^\epsilon$-carbamoyl Lys^6, Ilys^8, D-Ala^{10}]-GnRH, which is referred to as Antarelix.

U.S. Pat. No. 5,296,468, issued Mar. 22, 1994, discloses the design and synthesis of a number of GnRH antagonists wherein the side chains of selected residues are reacted to create cyanoguanidino moieties, some of which subsequently spontaneously convert to a desired heterocycle, e.g. a 3-amino-1,2,4-triazole(atz). Such cyanoguanidino moieties are built upon the omega-amino group in an amino acid side chain, such as lysine, ornithine, 4-amino phenylalanine (4Aph) or an extended chain version thereof, such as 4-amino homophenylalanine (4Ahp). GnRH antagonists having such significantly modified or unnatural amino acids in the 5- and 6-positions exhibit good biological potency, and those built upon Aph are generally considered to be preferred. One that is especially preferred is Azaline B, i.e. [Ac-D-2Nal^1, D-4ClPhe^2, D-3Pal^3, 4Aph(atz)^5, D-4Aph(atz)^6, ILys^8, D-Ala^{10}]-GnRH. U.S. Pat. No. 5,506,207 discloses biopotent GnRH antagonists wherein amino-substituted phenylalanine side chains of residues in the 5- and 6-positions are acylated; one particularly potent decapeptide is Acyline, i.e. [Ac-D-2Nal^1, D-4ClPhe^2, D-3Pal^3, 4Aph(Ac)^5, D-4Aph(Ac)^6, ILys^8, D-Ala^{10}]-GnRH.

Despite the attractive properties of this group of GnRH antagonists, the search has continued for still further improved GnRH antagonists, particularly those which exhibit long duration of biological action. It can frequently be important that a peptide analog should exhibit a long duration of activity with respect to LH secretion, a property which may be enhanced by the peptide's resistance to proteolytic enzyme degradation in the body for both short-term and long-term treatment indications. In addition, to facilitate administration of these compounds to mammals, particularly humans, without significant gelling, it is considered extremely advantageous for such GnRH antagonistic decapeptides to have high solubility in water at normal physiologic pH, i.e. about pH 5 to about pH 7.4.

SUMMARY OF THE INVENTION

It has now been found that certain other modifications to the 5-position residue, or the 5- and 6-position residues, in this subclass of GnRH antagonists, which includes Cetrorelix, Antarelix, Acyline, Antide and others, unexpectedly result in compounds which when administered sc exhibit the particularly advantageous property of long duration of bioactivity. These modifications are made to a residue of 4aminoPhe or its equivalent 4Ahp or to 4-aminomethyl phenylalanine (4Amf) wherein the primary amino group is bonded to a methyl group attached in the 4- or para-position. In such modifications, the amino group of the side chain is reacted with an isocyanate to form a urea group or reacted with a heterocyclic carboxylic acid containing at least 2 nitrogen atoms arranged to constitute a urea moiety. The preferred heterocyclic reactants are D- or L-hydroorotic acid (Hor)($C_4N_2H_5(O)_2COOH$) and D- or L-2-Imidazolidone-4-carboxylic acid (Imz)($C_3N_2H_5(O)(COOH)$.

Generally, GnRH antagonist decapeptides having the following formula, and closely related analogs and the pharmaceutically acceptable salts, are found to have improved pharmacological properties, particularly long duration of bioactivity:

X-D-2Nal-(A)D-Phe-D-3Pal-Ser-Xaa_5-Xaa_6-Leu-Xaa_8-Pro-Xaa_{10}

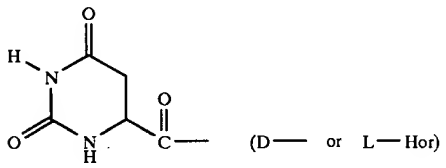
wherein:

X is an acyl group having up to 7 carbon atoms or Q, with Q being

and with R being H or lower alkyl;

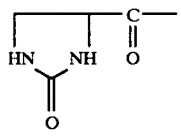
A is 4Cl, 4F, 4Br, $4NO_2$, $4CH_3$, $4OCH_3$, $3,4Cl_2$ or C^{α}Me4Cl;

Xaa_5 is 4Aph(Q_1) or 4Amf(Q_1) with Q_1 being Q or



(D— or L—Hor)

or



(D— or L—Imz)

Xaa_6 is D-4Aph(Q_2), D-4Amf(Q_2), D-Lys(Nic), D-Cit, D-Hci or D-3Pal, with Q_2 being For, Ac, 3-amino-1,2,4 triazole, or Q_1;

Xaa_8 is Lys(ipr), Arg, Har, Arg(Et_2) or Har(Et_2); and

Xaa_{10} is D-Ala-NH_2, $NHCH_2CH_3$, Gly-NH_2, AzaGly-NH_2, Ala-NH_2, Agl-NH_2, D-Agl-NH_2, Agl(Me)-NH_2 or D-Agl(Me)-NH_2.

Alternatively, when Xaa_6 contains Q or D- or L-Hor or D- or L-Imz, Xaa_5 may have Ac, For or 3-amino-1,2,4-triazole as Q_1.

These antagonists are particularly useful to suppress the secretion of gonadotropins and as fertility regulators in humans because they exhibit long duration of activity, the 3-position of the decapeptide. They have improved solubility in aqueous buffers at physiologic pHs and acceptable side effects with respect to stimulation of histamine release, i.e. better than the GnRH superagonists which are now being clinically used; they also exhibit minimal gelling upon subcutaneous(sc) injection at effective concentrations. These GnRH antagonists also perform well in an anaphylactoid

assay causing a relatively small wheal. As a result, these peptides find particular use in administration to mammals, especially humans, as fertility regulators and for the treatment of pathological conditions such as precocious puberty, hormone-dependent neoplasia, dysmenorrhea, endometriosis, steroid-dependent tumors, and the other short-term and long-term indications mentioned hereinbefore. They are also useful diagnostically.

Because these GnRH antagonists are readily soluble in the physiologic pH range of about 5 to about 7.4, they can be formulated and administered in concentrated form, particularly at a pH between about 5 and about 7. Because of their polar character, they are particularly suitable for use in slow-release preparations based upon known copolymers. Because these GnRH antagonists exhibit effective suppression of LH and FSH for long duration, they are also particularly effective for the contraceptive treatment of male mammals (with the administration of testoterone) and for the treatment of steroid-dependent tumors.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

During the last 10 to 12 years, the particular properties of each of the 10 residues in the sequence of GnRH, from the standpoint of creating an effective antagonist, have been studied in depth, and as a result of these studies, it has been discovered that there are various equivalent residues that can be chosen and that substitutions of one of these equivalents for another does not significantly detract from the biological potency of decapeptide GnRH antagonists. Such equivalent substitutions may be made in the GnRH antagonists of the present invention.

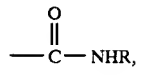
For example, it has become generally accepted that the inclusion of a para-substituted D-Phe or 2,4 dichloro-substituted D-Phe or D-C^{α}Me4ClPhe or D-pentamethyl (Me_5)Phe residue in the 2-position adds significantly to GnRH antagonist activity; however, the specific identity of the ring substituent is of only relatively minor importance when selected from among the following: chloro, fluoro, bromo, nitro, methyl and alkoxy. Therefore, such residues in the 2-position are considered to be the equivalent of D-4ClPhe which is commonly used therein. Phe^7 is considered to be equivalent to Leu^7. The N-terminus is preferably N-acylated, preferably by acetyl (Ac), but also by other acyl groups having up to 7 carbon atoms, e.g. formyl (For), acrylyl (Acr) n-propionyl (Pn), butyryl (Bt), valeryl (Vl), vinylacetyl (Vac) and benzoyl (Bz); alternatively, it may be modified by a substituted or unsubstituted carbamoyl. Other longer acyl groups are considered to be equivalents but are less preferred. The α-amino group on the 5-position residue may be optionally methylated, as disclosed in U.S. Pat. No. 5,110,904, to increase solubility in water, but such modification may result in a shortening of duration of LH suppression and in greater potential for histamine release. The C-terminus is preferably D-Ala-NH_2; however, Gly-NH_2, $NHCH_2CH_3$, AzaGly-NH_2, Ala-NH_2, Agl-NH_2, D-Agl-NH_2, Agl(Me)-NH_2 and D-Agl(Me)-NH_2 may instead be used as they are considered to be known equivalents.

As stated hereinbefore, the present invention is considered to provide a family of GnRH antagonists represented by the following formula:

X-D-2Nal-(A)D-Phe-D-3Pal-Ser-Xaa_5-Xaa_6-Leu-Xaa_8-Pro-Xaa_{10} and the pharmaceutically acceptable salts thereof wherein:

X is For, Ac, Acr, Pn, Bt, Vl, Vac, Bz or Q,

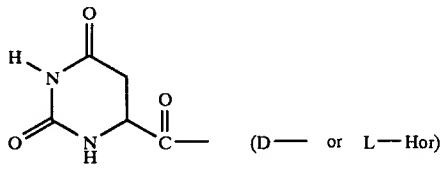
with Q being



and with R being H or lower alkyl;

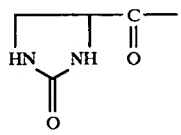
A is 4Cl, 4F, 4Br, $4NO_2$, $4CH_3$, $4OCH_3$, $3,4Cl_2$ or C^{α}Me4Cl;

Xaa_5 is 4Aph(Q_1) or 4Amf(Q_1) with Q_1 being Q or



(D— or L—Hor)

or



(D— or L—Imz)

Xaa_6 is D-4Aph(Q_2), D-4Amf(Q_2), D-Lys(Nic), D-Cit, D-Hci or D-3Pal, with Q_2 being For, Ac, 3-amino-1,2,4 triazole, or Q_1;

Xaa_8 is Lys(ipr), Arg, Har, Arg(Et_2) or Har(Et_2); and

Xaa_{10} is D-Ala-NH_2, $NHCH_2CH_3$, Gly-NH_2, AzaGly-NH_2, Ala-NH_2, Agl-NH_2, D-Agl-NH_2, Agl(Me)-NH_2 or D-Agl(Me)-NH_2.

In a closely related family of GnRH antagonists, Xaa_5 may have either Ac, For or 3-amino-1,2,4-triazole as Q_1, in which case Xaa_6 includes Q_2 in the form of Q or D- or L-Hor or D- or L-Imz.

By D-Nal is meant the D-isomer of alanine which is substituted by naphthyl on the β-carbon atom, i.e., also referred to as 3-D-Nal. Preferably D-2Nal is employed wherein the attachment to naphthalene is at the 2-position on the ring structure; however, D-1Nal may also be used. D-Cpa represents chloro-D-Phe, and D-4ClPhe, i.e. D-4Cpa, is preferred. D-Pal represents the D-isomer of alanine which has been substituted by pyridyl on the β-carbon atom; preferably, the linkage is to the 3-position on the pyridine ring, i.e. D-3Pal (β-3-pyridyl-D-Ala), although D-2Pal(β-2-pyridyl-D-Ala) might instead be used. By 4Aph is meant $4NH_2$Phe wherein the amino substituent on the phenyl ring is at the 4-position; $3NH_2$Phe(3Aph) and $4NH_2$-homophenylalanine (4Ahp) are considered to be its equivalents in these analogs. Moreover, it is believed that $2NH_2$Phe is also equivalent from the standpoint of biopotency. By 4Amf is meant $4NH_2CH_2$Phe where there is a methylene linkage to the side chain amino group; $3NH_2CH_2$Phe(3Amf) is considered equivalent. By Hor is meant L-hydroorotyl, and by Imz is meant L-2-imidazolidone-4-carbonyl—either of which may also be used as the D-isomer or the D/L mixture. By atz is meant 3-amino-1,2,4-triazole. Aph(atz) is also known by the more precise chemical name 4-(3'-amino-1H-1', 2',4'-triazoyl-5'-yl) amino phenylalanine. By Lys (Nic) is meant N^{ϵ}-nicotinoyl lysine, i.e. the ϵ-amino group of Lys is acylated with 3-carboxypyridine. By D-Cit is meant the D-isomer of citrulline, and by D-Hci is meant the D-isomer of homocitrulline, which is also D-N^{ϵ}-carbamoyl lysine. By ILys or Lys(ipr) is meant N^{ϵ}-isopropyl lysine where the ϵ-amino group of Lys is alkylated. By AzaGly-

NH_2 is meant $NHNHCONH_2$. By Dbu is meant alpha, gamma-diamino butyric acid, and by Dpr is meant α,β-diamino propionic acid. By Har is meant homoarginine. By Agl is meant α-aminoglycine. By Cbm is meant carbamoyl, and by MeCbm is meant methylcarbamoyl or —$CONHCH_3$. By lower alkyl is meant C_1 to C_5, preferably C_1 to C_3, and more preferably C_1 or C_2, i.e. methyl(Me) or ethyl(Et).

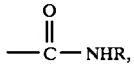
Although the preferred D-isomers for incorporation in the 6-position of these GnRH antagonists are specifically disclosed, it should be understood that as a result of the extensive research in the field over the past two decades, there are many known equivalent D-isomers. Such prior art D-isomer substitutions may be compatible and not detract from the biopotency afforded by the specific 5-position substitutions disclosed herein, and may optionally be utilized.

A preferred subgenus of GnRH antagonists has the formula:

X-D-2Nal-(A)D-Phe-D-3Pal-Ser-Xaa_5-Xaa_6-Leu-Lys (ipr)-Pro-Xaa_{10} and the pharmaceutically acceptable salts thereof wherein:

X is For, Ac, Acr, Pn, Bt, Vl, Vac, Bz or Q,

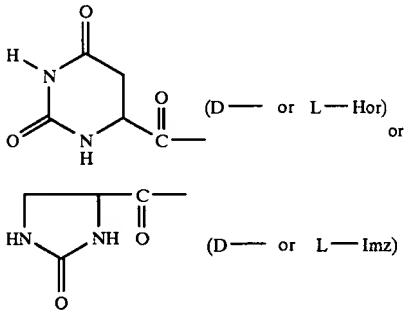
with Q being



and with R being H or lower alkyl,

A is 4Cl or 4F;

Xaa_5 is 4Aph(Q_1) or 4Amf(Q_1) with Q_1 being



Xaa_6 is D-4Aph(Q_2), D-4Amf(Q_2), D-Cit, D-Lys(Nic) or D-3Pal, with Q_2 being For, Ac, Q or Q_1; and

Xaa_{10} is D-Ala-NH_2, $NHCH_2CH_3$ or Gly-NH_2.

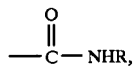
An additional preferred subgenus of GnRH antagonists has the formula:

X-D-2Nal-D-4ClPhe-D-3Pal-Ser-Xaa_5-Xaa_6-Leu-Lys (ipr)-Pro-D-Ala-NH_2 and the pharmaceutically acceptable salts thereof

wherein:

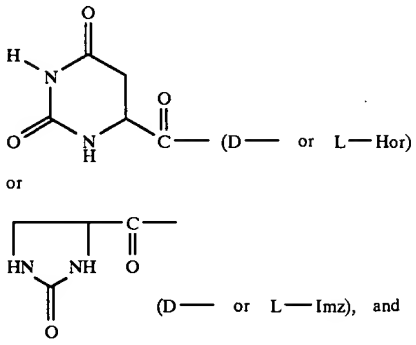
X is Ac or Q,

with Q being



and with R being H or methyl;

Xaa_5 is 4Aph(Q_1) or 4Amf(Q_1) with Q_1 being

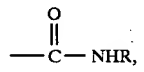


Xaa_6 is D-4Aph(Q_2), D-4Amf(Q_2) or D-3Pal, with Q_2 being Ac, Q or Q_1.

Another preferred subgenus of GnRH antagonists has the formula:

MeCbm-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(Hor)-D-Xaa_6-Leu-ILys-Pro-Xaa_{10} and the pharmaceutically acceptable salts thereof wherein D-Xaa_6 is D-4Amf (Q_1), D-4Aph(Q_1) or D-3Pal,

with Q_1 being D-Hor or



and with R being H or lower alkyl, and preferably H or methyl; and wherein Xaa_{10} is D-Ala-NH_2 or an equivalent.

The compounds of the present invention can be synthesized by classical peptide solution synthesis, and such synthesis is preferred for large quantities of product. To obtain limited quantities, e.g. less than 1 kg, it may be preferable to synthesize them using a solid phase technique. A chloromethylated resin or a hydroxymethylated resin may be used; however, a methylbenzhydrylamine(MBHA) resin, a benzhydrylamine (BHA) resin or some other suitable resin known in the art which directly provides a C-terminal amide upon cleavage is preferably employed. Should equivalent peptides having a substituted amide at the C-terminus be desired, they are preferably synthesized using an N-alkylamino methyl resin as taught in U.S. Pat. No. 4,569,967, issued Feb. 11, 1986. Solid phase, chain elongation synthesis is usually conducted in a manner to stepwise add individual amino acids to the chain, e.g. in the manner set forth in detail in the U.S. Pat. No. 5,296,468. Side-chain protecting groups, as are well known in the art, are preferably included as a part of any amino acid which has a particularly reactive or labile side chain when it is being coupled into the chain being built upon the resin. Such synthesis provides a fully protected intermediate peptidoresin.

One example of a chemical intermediate, which might be used to synthesize a GnRH antagonist having a desired residue in the 5- and 6-positions containing hydroorotyl or the like is represented by the formula: X^1-D-2Nal-D-4ClPhe-D-3Pal-Ser(X^2)-4Aph (X^3)-D-4Aph(X^3)-Leu-ILys (X^4)-Pro-X^5. In synthesizing peptide intermediates having this formula and other analogs, groups X^1 to X^5 as set forth hereinafter may be employed.

X^1 is an α-amino protecting group of the type known to be useful in the art in the stepwise synthesis of polypeptides and when X in the desired peptide composition is a particu-

lar acyl group, that group may be used as the protecting group. Among the classes of α-amino protecting groups covered by X^1 are (1) acyl-type protecting groups, such as formyl(For), trifluoroacetyl, phthaloyl, p-toluenesulfonyl (Tos), benzoyl(Bz), benzenesulfonyl, dithiasuccinoyl(Dts) o-nitrophenylsulfenyl(Nps), tritylsulfenyl, o-nitrophenoxyacetyl, acrylyl(Acr), chloroacetyl, acetyl(Ac) and γ-chlorobutyryl; (2) aromatic urethan-type protecting groups, e.g., benzyloxycarbonyl(Z), fluorenylmethyloxycarbonyl(Fmoc) and substituted benzyloxycarbonyl, such as p-chlorobenzyloxycarbonyl (ClZ), p-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl and p-methoxybenzyloxycarbonyl; (3) aliphatic urethan protecting groups, such as tertbutyloxycarbonyl(Boc), diisopropylmethoxycarbonyl, isopropyloxycarbonyl, ethoxycarbonyl and allyloxycarbonyl; (4) cycloalkyl urethan-type protecting groups, such as cyclopentyloxycarbonyl, adamantyloxycarbonyl and cyclohexyloxycarbonyl; (5) thiourethan-type protecting groups, such as phenylthiocarbonyl; (6) alkyl-type protecting groups, such as allyl(Aly), triphenylmethyl(trityl) and benzyl(Bzl); (7) trialkylsilane groups, such as trimethylsilane. The preferred α-amino protecting group is Boc.

X^2 is a protecting group for the hydroxyl side chain of Ser, e.g. Ac, Bz, trityl, DCB or benzyl ether(Bzl) and is preferably Bzl.

$X^3$is a protecting group for a side chain amino group which is not removed when the α-amino protecting group or another amino-protecting group is removed. Illustrative examples include (1) base-labile groups, such as Fmoc, or some other weak-acid stable, aromatic urethane-type protecting group; (2) thiol-labile groups, such as dithiasuccinoyl(Dts) which may be removed or cleaved by thiolysis; (3) hydrazine-labile groups, such as phthaloyl(Pht) which is cleaved by hydrazinolysis; (4) nucleophile-labile groups, such as o-nitrophenylsulfenyl(Nps) and the like which are cleaved by thioacetamide or by weak acids or their salts; (5) photolabile groups which are cleaved by photolysis; and (6) groups selectively removable by reduction, such as Dts. Fmoc is preferred for a Boc SPPS strategy.

X^4 is an acid-labile protecting group for a primary or secondary amino side chain group, such as Z or 2ClZ.

X^5 may be D-Ala-, Gly-, Ala-, Agl-, D-Agl-, Agl(Me)- or D-Agl(Me)-NH-[resin support], or N(Et)-[resin support]; X^5 may also be an amide either of Gly or Ala or D-Ala, or a lower alkyl-substituted amide attached directly to Pro, or AzaGly-NH_2.

The criterion for selecting side chain protecting groups X^2 through X^4 is that the protecting group should generally be stable to the reagent under the reaction conditions selected for removing the α-amino protecting group (preferably Boc) at each step of the synthesis. These protecting groups generally should not be split off under coupling conditions but should be removable upon completion of the synthesis of the desired amino acid sequence under reaction conditions that will not alter the peptide chain. The protecting groups initially employed for the 5- and 6-position residues are preferably removed and selective reactions are carried out prior to cleavage of the ultimate peptide from the resin, as explained hereinafter. If a decapeptide intermediate is synthesized as set forth hereinbefore, the X^3 protecting groups may be desirably individually removable.

When the X^5 group is D-Ala-NH-[resin support], an amide bond connects D-Ala to a BHA resin or to a MBHA resin; this is likewise the case when Agl or D-Agl is used at the C-terminus. When X^5 is N(Et)-[resin support], an ethylamide bond connects Pro to an N-alkylaminomethyl resin (NAAM).

When the N-terminus is to be acetylated, for example, it is possible to employ acetyl as the X^1 protecting group for the α-amino group of β-D-Nal in the 1-position by adding it to the amino acid before it is coupled to the peptide chain; however, a reaction is preferably carried out with the peptide intermediate on the resin. After deblocking the α-amino group and while desired side chain groups remain protected, acetylation is preferably carried out by reacting with acetic anhydride, alternatively reaction can be carried out with acetic acid, in the presence of diisopropyl or dicyclohexyl carbodiimide (DIC or DCC), or by some other suitable acylation reaction as known in the art. A similar procedure is carried out when a carbamoyl or substituted carbamoyl group is desired at the N-terminus. When the deprotected side chain amino groups are modified while the residue is part of the peptide chain, the reaction may be carried out using an appropriate isocyanate in the presence of an appropriate base, for example, N,N-diisopropylethylamine (DIEA), although the use of such a base is optional. When an unsubstituted carbamoyl group is desired in the final product, the deprotected amino side chain may be reacted with benzyl isocyanate, p-tosyl isocyanate, trimethylsilyl isocyanate or tert-butyl isocyanate, with the latter being preferred. Using such a strategy, the t-butyl moiety is removed during deprotection, leaving the carbamoyl group.

The invention also provides a novel method for making such a GnRH antagonist having, for example, the formula: Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(Hor)-D-4Aph(Ac)-Leu-ILys-Pro-D-Ala-NH_2, which method comprises (a) forming an intermediate peptide having the formula: Boc-D-4Aph(X^3)-Leu-ILys(X^4)-Pro-X^5 wherein X^3 is a base-labile, hydrazine-labile or other appropriately labile protecting group for an amino group; X^4 is an acid-labile protecting group for an amino side chain; and X^5 is D-Ala-NH-[resin support]; (b) removing X^3 from D-4Aph to deprotect the side chain primary amino group of this amino acid residue of the intermediate peptide; (c) reacting this deprotected side chain primary amino group with acetic anhydride; (d) completing the chain elongation to create the intermediate X^1-D-2Nal-D-4Cpa-D-3Pal-Ser(X^2)-4Aph(X^3)-D-4Aph(Ac)-Leu-Ilys(X^4)-Pro-X^5, wherein X^1 is hydrogen or an α-amino protecting group and X^2 is hydrogen or a protecting group for a hydroxyl group of Ser; (e) deblocking the α-amino group at the N-terminus and acetylating; (f) removing X^3 from 4Aph and reacting the deprotected primary amino group with hydroorotic acid; and (g) splitting off any remaining protecting groups and/or cleaving from resin support included in X^5.

Final purification of the peptide is effected by chromatography and preferably by using RP-HPLC, as known in the art, see J. Rivier, et al. *J. Chromatography,* 288, 303–328 (1984), and C. Miller and J. Rivier, *Biopolymers* (Peptide Science), 40, 265–317 (1996).

The GnRH antagonists of the invention are considered to be effective at levels of less than 100 micrograms per kilogram of body weight, when administered subcutaneously at about noon on the day of proestrus, to prevent ovulation in female rats. For prolonged suppression of ovulation, it may be necessary to use dosage levels in the range of from about 0.1 to about 2.5 milligrams per kilogram of body weight. The antagonists are also effective to arrest spermatogenesis when administered to male mammals on a regular basis and can thus be used as contraceptives. Because these compounds will reduce testosterone levels and thus libido (an undesired consequence in the normal, sexually active male), it may be desirable to administer replacement dosages of testosterone along with the GnRH

antagonist in order to achieve azoospermia or hair growth while maintaining libido. These antagonists can also be used to regulate the production of gonadotropins and sex steroids and for other of the long-term and short-term indications as indicated hereinbefore, and they can be used in veterinary applications as contraceptives for pets.

The peptides provided by the invention are particularly soluble at physiological pHs and can be prepared as relatively concentrated solutions for administration, particularly for subcutaneous injection. These peptides are well-tolerated in the body and do not tend to gel when administered subcutaneously at effective concentrations. Generally pharmaceutical compositions including such peptides and a suitable pharmaceutically acceptable excipient can be administered iv, ip, subcutaneously or the like at levels of between about 0.001 mg to about 2.5 mgs per Kg of body weight per day, with 0.5 mg/kg/day usually being sufficient.

The appropriately protected D- or L-hydroorotyl-containing, carbamoyl-containing and/or D- or L-imidazolidone-carbonyl-containing amino acids are preferably synthesized and then employed in a chain elongation peptide synthesis. However, synthesis may also be effected by initially incorporating an appropriately protected 4Aph, 4Ahp, 4Amf or Dpr residue at the desired position in the peptide intermediate, and this may be the laboratory method of choice where only small quantities are initially desired. This strategy is accomplished by subsequently deprotecting the particular residue (either immediately or subsequently during the synthesis) and then reacting the deprotected side chain amino group with the desired reagent.

The present invention is further described by the examples which follow. Such examples, however, are not to be construed as limiting in any way either the spirit or the scope of the present invention. The following examples illustrate GnRH antagonists embodying various features of the invention, and all of these compounds include at least one D-isomer amino acid residue.

EXAMPLE 1

The peptide having the formula: Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-Lys(Nic)-D-Lys(Nic)-Leu-ILys-Pro-D-Ala-NH_2 (Antide) has been found to exhibit very good biological properties as a GnRH antagonist, as has the peptide which is presently referred to as Acyline and which differs from Antide in only the 5- and 6-positions. It has now been found that by using these molecules as a starting point and by making other substitutions in the 5- and 6-positions or in the 5-position of the decapeptide Acyline, GnRH antagonists having improved duration of bioactivity in vivo are obtained. With respect to positions 1–4 and 7–10, it is noted that Antide, Acyline and Azaline are all exactly the same.

The following decapeptide [4Aph(Hor)^5, D-4Aph(Cbm)^6]-Antide or [Ac-D-2Nal^1, D-4Cpa^2, D-3Pal^3, 4Aph(Hor)^5, D-4Aph(Cbm)^6, ILys^8, D-Ala^{10}]-GnRH (Peptide No. 1) is synthesized by solid-phase synthesis. This peptide has the following formula: Ac-D-2Nal-(4Cl)D-Phe-D-3Pal-Ser-4Aph(hydroorotyl)-D-4Aph(carbamoyl)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2.

About 0.50 gram (0.54 mmol/g) of MBHA resin (Bachem) are initially used, and Boc-protected D-Ala is coupled to the resin over about a 2-hour period in dimethylformamide(DMF)/CH_2Cl_2 using about 0.65 millimole of Boc derivative and diisopropylcarbodiimide(DIC) and anhydrous 1-hydroxybenzotriazole (HOBt) as activating or coupling reagents. The D-Ala residue attaches to the MBHA residue by an amide bond.

Following the coupling of each amino acid residue, washing, deblocking and then coupling of the next amino acid residue are carried out in accordance with the following manual synthesis schedule for about 0.5 to 1 gram of starting resin:

STEP	REAGENTS AND OPERATIONS	MIX TIMES MIN.
1	Methanol (MeOH) wash-15 ml. (2 times)	1
2	CH_2Cl_2 wash-30 ml. (3 times)	1
3	50% TFA plus 1% m-cresol in CH_2Cl_2-25 ml. (2 times)	5, 20
4	Isopropyl alcohol wash-20 ml. (2 times)	1
5	TEA 10% in CH_2Cl_2-20 ml. (2 times)	2
6	MeOH wash-15 ml. (2 times)	1
7	CH_2Cl_2 wash-20 ml. (3 times)	1
8	Boc-amino acid (0.5–1.0 mmole) and HOBt (0.5–1.0 mmole) in 10–20 ml. of dimethylformamide(DMF): DCM or N-methylpyrrolidone (NMP): DCM, depending upon the solubility of the particular protected amino acid, plus DIC or DCC (0.5–1.0 mmole) in CH_2Cl_2	1–17 hours
9	MeOH wash-15 ml. (2 times)	1
10	DCM wash-20 ml. (3 times)	1

The above schedule is used for coupling of each of the amino acids of the peptide of the invention after the first amino acid has been attached. N^{α}-Boc protection is used for each of the amino acids coupled throughout the synthesis. N^{α}Boc-β-D-2Nal is prepared by a method known in the art, e.g. as described in detail in U.S. Pat. No. 4,234,571, issued Nov. 18, 1980; it is also commercially available from SyntheTech, Oregon, U.S.A. The side chain primary amino groups of 4Aph in the 5-position and of D-4Aph in the 6-position are protected by Fmoc. Benzyl ether (Bzl) is preferably used as a side chain protecting group for the hydroxyl group of Ser; however, Ser may be coupled without side chain protection. N^{α}Boc-Lys(Ipr,Z) is used for the 8-position residue.

After adding D-4Aph for the 6-position residue as N^{α}Boc-D-4Aph(Fmoc), the following intermediate is present: Boc-D-4Aph(Fmoc)-Leu-Lys(Ipr,Z)-Pro-D-Ala-NH-[MBHA resin support]. The side chain amino group on the 6-position residue is then modified after first removing the side-chain protection. The Fmoc protecting group is removed by successive treatments with 25 percent piperidine in DMF (10 ml) for about 15 minutes each. After preferably washing the peptidoresin with DMF, the newly freed amino group is treated with a 20-fold excess of tert-butyl isocyanate in DMF at room temperature for about 12 hours, or until complete as checked using a ninhydrin test. The peptidoresin is then subjected to the standard wash. When Boc is removed in order to add the next residue, some t-butyl moiety is also removed.

The 5-position residue is then added as N^{α}Boc-4Aph (Fmoc). Its side chain is then deprotected as before, and a reaction is carried out with 0.10 g (0.66 mmol) of L-hydroorotic acid, 90 mg (0.66 mmol) of HOBt and 0.66 mmol of DIC in 3 ml of DMF at room temperature for about 8 hours, or until complete as checked using a standard ninhydrin test. After washing and N^{α}Boc removal, the synthesis of the decapeptide is completed by sequentially reacting with N^{α}Boc-Ser(Bzl), N^{α}Boc-D-3Pal, N^{α}Boc-4ClD-Phe, and N^{α}Boc-β-D-2Nal.

After deblocking the α-amino group at the N-terminus using trifluoroacetic acid (TFA), acetylation is achieved using a large excess of acetic anhydride in dichloromethane (DCM) for about 30 minutes. Alternatively, the Fmoc protection of 4Aph is not removed until after the acetylation of

the N-terminus, and the reaction with hydroorotic acid is then carried out.

The peptidoresin is dried, and then cleavage of the peptide from the resin and deprotection of the Ser and the Lys side chains are carried out at about 0° C. with 15 ml of HF for about 1.5 hours. Anisole (0.5 ml.) is added as a scavenger prior to HF treatment. After the removal of HF under vacuum, the resin is washed twice with 100 ml. of ethyl ether. The cleaved peptide is extracted with 0.2% TFA in 25% CH_3CN/H_2O, repeating the process and using 100 ml. each time. The extracts are pooled and lyophilized, and they provide about 600 mg of a crude peptide powder.

Purification of the peptide is then effected by preparative high performance liquid chromatography (HPLC), as known in the art and specifically set forth in J. Rivier, et al. *J. Chromatography*, 288, 303–328 (1984). The first preparative RP-HPLC separation uses a TEAP (triethylammonium phosphate) buffer system, and a final separation is carried out using a 0.1% TFA (trifluoroacetic acid) gradient, all as described in detail in the *J. Chromatography* article.

The peptide (about 30 mg) (hereinafter referred to as Peptide No. 1) is judged to be homogeneous using capillary zone electrophoresis (CZE), and the purity is estimated to be about 98%. Amino acid analysis of the resultant, purified peptide is consistent with the formula for the prepared structure. Liquid secondary ion mass spectrometry (LSIMS) measures molecular weight as 1631.9 Da which is consistent with the expected mass of 1631.8 Da for this peptide.

Hydrophilicity is tested by measuring retention time using RP-HPLC with a gradient of 40% Buffer B to 70% Buffer B over 30 minutes, with Buffer A being TEAP pH 7.0 and Buffer B being 70% CH_3CN and 30% Buffer A. Peptide No. 1 is more hydrophilic than Acyline, eluting earlier than Acyline. Its solubility in aqueous buffers at a pH of from about 5 to about 7 and its resistance to in vivo gelling, along with a long-acting biopotency to suppress circulating LH levels as described hereinafter, render it particularly suitable for administration by subcutaneous injection compared to other compounds of generally comparable biological efficacy.

The peptide is assayed in vivo to determine its effectiveness to suppress the secretion of LH in rats. Measurement of circulating LH levels in castrated male Sprague-Dawley rats treated subcutaneously with the peptide is carried out as reported in C. Rivier et al. *Biol. Reproduc.*, 1983, 29, 374–378. The peptides are first dissolved at a concentration of 1.0 or 10 mg/ml in bacteriostatic water and then further diluted in 0.04 M phosphate buffer containing 0.1% BSA. Subsequent dilutions are made in phosphate buffer. The peptides are injected sc into 5 rats, and blood samples (300 μl) are collected under metotane anesthesia. Sera (50 μl) are tested for LH levels in duplicate using reagents provided by the National Pituitary and Hormone Distribution Program of the NIDDK. Testing shows that a dosage of 50 μg of peptide per rat suppresses LH secretion to levels that are far less than 50% of control levels throughout the 96-hour period following injection. Moreover, the levels measured after 96 hours are about only 30% of the LH levels exhibited by rats similarly injected with a dose of 50 micrograms of Acyline. Peptide No. 1 is considered to be very long-acting. Examination of the rats shows that the peptide was very well tolerated, with no significant gelling at the point of injection being detectable.

Experience gained from the testing of a large number of GnRH antagonists shows that a peptide exhibiting such long-acting suppression of LH would, if assayed in vivo in mature female Sprague-Dawley rats, fully block ovulation at a dosage of 2.5 micrograms.

EXAMPLE 1A

The synthesis set forth in Example 1 is repeated, substituting N^{α}Boc-D-4Amf(Fmoc) for N^{α}Boc-D-4Aph(Fmoc). Following deprotection of the D-4Amf side chain, reaction with t-butyl isocyanate is carried out as before. Cleavage from the resin and deprotection, followed by purification, are carried out as described in Example 1. The peptide Ac-β-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(hydroorotyl)-D-4Amf(carbamoyl)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification and judged to be homogeneous, with its purity estimated to be greater than 99 percent. MS analysis shows a mass of 1645.9 Da which compares favorably to the expected mass of 1645.8 Da. From the HPLC results,it can be seen that this peptide is more hydrophilic than Acyline.

Assaying this peptide in the standard in vivo rat LH Suppression test shows that, at a dosage of 50 micrograms, it is as effective as Acyline in suppressing LH levels at 1, 2 and 3 days. At 96 hours, the LH levels are only about 25% of those of the rats injected with Acyline. Peptide No. 1A is considered to be very long-acting.

EXAMPLE 1B

To form the analog [4Aph(Hor)^5]-Acyline, the synthesis set forth in Example 1 is repeated substituting acetic anhydride for t-butyl isocyanate for the reaction with the deprotected position-6 side-chain. Cleavage of the decapeptide from the resin and deprotection, followed by purification, are carried out as described in Example 1. The peptide Ac-β-D-2Nal-D-Cpa-D-3Pal-Ser-4Aph(hydroorotyl)-D-4Aph(acetyl)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 99 percent. MS analysis shows a mass of 1630.6 Da, which is in agreement with the calculated mass of 1630.8 Da.

Assaying this peptide in the standard in vivo rat test as in Example 1, shows that, at a dosage of 50 micrograms, the peptide is bioactive and about as effective as Acyline at day 1 through day 4. It is considered to exhibit very long-acting duration for the suppression of LH.

This synthesis is repeated substituting N^{α}Boc-D-4Amf (Fmoc) for N^{α}Boc-D-4Aph(Fmoc) to create the decapeptide [4Aph(Hor)^5, D-Amf(Ac)^6]-Antide, which is generally as biopotent in the suppression of secretion of LH.

EXAMPLE 1C

The synthesis set forth in Example 1B is repeated substituting D/L hydroorotic acid for L-hydroorotic to form the similar decapeptide. Cleavage from the resin and deprotection, followed by purification, are carried out as described in Example 1. The peptide Ac-β-D-2Nal-D-Cpa-D-3Pal-Ser-4Aph(D/L-hydroorotyl)-D-4Aph(acetyl)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be a homogeneous mixture of two compounds without other impurities. MS analysis shows a mass of 1630.6 Da, which is in agreement with the calculated mass of 1630.8 Da.

Assaying this peptide in the standard in vivo rat test as in Example 1, shows that, at a dosage of 50 micrograms, the peptide is bioactive and about as effective as Acyline at day 1 through day 4. It is considered to exhibit long-acting duration for the suppression of LH.

EXAMPLE 1D

The synthesis set forth in Example 1B is repeated substituting D-hydroorotic acid for L-hydroorotic to form the similar decapeptide. Cleavage from the resin and deprotection, followed by purification, are carried out as described in Example 1. The peptide Ac-β-D-2Nal-D-Cpa-D-3Pal-Ser-4Aph(D-hydroorotyl)-D-4Aph(acetyl)-Leu-Lys (isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC

purification. It is judged to be homogeneous, and its purity is estimated to be greater than 98 percent. MS analysis shows a mass of 1630.8 Da, which is in agreement with the calculated mass of 1630.8 Da.

Assaying this peptide in the standard in vivo rat test as in Example 1, shows that, at a dosage of 50 micrograms, the peptide exhibits long-acting duration of bioactivity for the suppression of LH, being about as effective as Acyline at day 1 through day 4.

EXAMPLE 1E

The synthesis set forth in Example 1B is repeated substituting N^{α}Boc-D-4FPhe for N^{α}Boc-D-4ClPhe to form the decapeptide [D-4FPhe^2, 4Aph(Hor)^5]-Acyline. Cleavage from the resin and deprotection, followed by purification, are carried out as described in Example 1. The peptide Ac-β-D-2Nal-D-4Fpa-D-3Pal-Ser-4Aph(hydroorotyl)-D-4Aph(acetyl)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 99 percent. MS analysis shows a mass of 1615.1 Da, which is in agreement with the calculated mass of 1614.8 Da.

Assaying this peptide in the standard in vivo rat test as in Example 1, shows that, at a dosage of 50 micrograms, the peptide is bioactive and about as effective as Acyline at day 1 through day 4. It is considered to exhibit long-acting duration for the suppression of LH.

EXAMPLE 1F

The synthesis set forth in Example 1B is repeated substituting N^{α}Boc-4Amf(Fmoc) for N^{α}Boc-4Aph(Fmoc) to form the decapeptide [4Amf(Hor)^5]-Acyline. Cleavage from the resin and deprotection, followed by purification, are carried out as described in Example 1. The peptide Ac-β-D-2Nal-D-4Cpa-D-3Pal-Ser-4Amf(hydroorotyl)-D-4Aph (acetyl)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 98 percent. MS analysis shows a mass of 1644.7 Da, which is in agreement with the calculated mass of 1644.8 Da.

Assaying this peptide in the standard in vivo rat test as in Example 1, shows that, at a dosage of 50 micrograms, the peptide is bioactive and about as effective as Acyline at 1 day through four days. It is considered to exhibit long-acting duration for the suppression of

EXAMPLE 1G

The synthesis set forth in Example 1 is repeated; however, instead of reacting the side chain amino of D-4Aph with t-butyl isocyanate, it and the 4Aph residue are simultaneously reacted with hydroorotic acid to form the decapeptide [4Aph(Hor^5), D-4Aph(Hor)$_6$]-Antide. Cleavage from the resin and deprotection, followed by purification, are carried out as described in Example 1. The peptide Ac-β-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(hydroorotyl)-D-4Aph (hydroorotyl)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 99 percent. MS analysis shows a mass of 1728.4 Da, which is in agreement with the calculated mass of 1728.8 Da. The results of the RP-HPLC show that this peptide is more hydrophilic than Azaline B which is in turn more hydrophilic than Acyline.

Assaying this peptide in the standard in vivo rat test as in Example 1, shows that, at a dosage of 50 micrograms, the peptide is bioactive and about as effective as Acyline at day 1 through day 4. It is considered to exhibit long-acting duration for the suppression of LH.

This synthesis is repeated substituting N^{α}Boc-D-4Amf (Fmoc) for N^{α}Boc-D-4Aph(Fmoc) to create the decapeptide [4Aph(Hor)^5, D-Amf(Hor)^6]-Antide, which is generally as biopotent in the suppression of secretion of LH.

EXAMPLE 1H

The synthesis set forth in Example 1 is repeated; however, instead of reacting the side chain amino of D-4Aph with t-butyl isocyanate, it is reacted with D-hydroorotic acid to form the decapeptide [4Aph(Hor^5), D-4Aph(D-Hor)^6]-Antide. Cleavage from the resin and deprotection, followed by purification, are carried out as described in Example 1. The peptide Ac-β-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph (hydroorotyl)-D-4Aph(D-hydroorotyl)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 98 percent. MS analysis shows a mass of 1728.7 Da, which is in agreement with the calculated mass of 1728.8 Da. The results of the RP-HPLC show that this peptide is more hydrophilic than Azaline B which is in turn more hydrophilic than Acyline.

Assaying this peptide in the standard in vivo rat test as in Example 1, shows that, at a dosage of 50 micrograms, the peptide is bioactive and about as effective as Acyline at day 1 through day 3. It is substantially more effective than Acyline at 4 days and is considered to exhibit very long-acting duration for the suppression of LH.

EXAMPLE 1J

The synthesis of the decapeptide [MeCbm-D-2Nal^1, 4Aph(Hor)^5]-Acyline is carried out by generally proceeding as set forth in Example 1B; however, instead of immediately removing the Fmoc-protecting group after adding N^{α}Boc-4Aph(Fmoc), the synthesis of the decapeptide on the resin is completed. Then, after deblocking the N-terminus, instead of reacting with acetic anhydride, a reaction is carried out with methyl isocyanate to form the methylcarbamoyl at the N-terminus. Then, the Fmoc is removed and the side chain amino of 4Aph is reacted with L-hydroorotic acid as in Example 1B. Cleavage from the resin and deprotection, followed by purification, are carried out as described in Example 1. The peptide methylcarbamoyl-β-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(hydroorotyl)-D-4Aph(acetyl)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be about 99 percent. MS analysis shows a mass of 1645.7 Da, which is in agreement with the calculated mass of 1645.8 Da. The results of the RP-HPLC show that this peptide is more hydrophilic than Azaline B which is in turn more hydrophilic than Acyline.

Assaying this peptide in the standard in vivo rat test as in Example 1, shows that, at a dosage of 50 micrograms, the peptide is bioactive and about as effective as Acyline at day 1 through day 3 and more effective by nearly 50% after 96 hours. It is considered to exhibit very long-acting duration for the suppression of LH.

EXAMPLE 1K

The synthesis set forth in Example 1 is repeated substituting N^{α}Boc-D-3Pal for N^{α}Boc-D-4Aph(Fmoc) to form the decapeptide [4Aph(Hor)^5, D-3Pal^6]-Antide. Cleavage from the resin and deprotection, followed by purification, are carried out as described in Example 1. The peptide Acetyl-β-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(hydroorotyl)-D-3Pal-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 99 percent. MS analysis shows a mass of 1574.7 Da, which is in agreement with the calculated mass of 1574.7 Da.

Assaying this peptide in the standard in vivo rat test as in Example 1, shows that, at a dosage of 50 micrograms, the peptide is bioactive and about as effective as Acyline through three days; however, after 96 hours, it exhibits suppression of LH level to values about 35% of those of Acyline. It is considered to exhibit very long-acting duration for the suppression of LH.

EXAMPLE 1L

The synthesis set forth in Example 1G is repeated substituting t-butyl isocyanate for hydroorotic acid to form the decapeptide [4Aph(Cbm)^5, D-4Aph(Cbm)^6]-Antide. Cleavage from the resin and deprotection, followed by purification, are carried out as described in Example 1. The peptide Ac-β-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph (carbamoyl)-D-4Aph(carbamoyl)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 99 percent. MS analysis shows a mass of 1534.9 Da, which is in agreement with the calculated mass of 1534.7 Da.

Assaying this peptide in the standard in vivo rat test as in Example 1, shows that, at a dosage of 50 micrograms, the peptide is bioactive and about as effective as Acyline through four days. It is considered to exhibit long-acting duration for the suppression of LH.

EXAMPLE 1M

The synthesis set forth in Example 1G is repeated substituting methyl isocyanate for hydroorotic acid to form the decapeptide [4Aph(MeCbm)^5, D-4Aph(MeCbm)^6]-Antide. Cleavage from the resin and deprotection, followed by purification, are carried out as described in Example 1. The peptide Ac-β-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph (methylcarbamoyl)-D-4Aph(methylcarbamoyl)-Leu-Lys (isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 99 percent. MS analysis shows a mass of 1562.8 Da, which is in agreement with the calculated mass of 1562.8 Da.

Assaying this peptide in the standard in vivo rat test as in Example 1, shows that, at a dosage of 50 micrograms, the peptide is bioactive and about as effective as Acyline for two days and then begins to drop off somewhat in its suppression of LH.

EXAMPLE 1P

Using the synthesis as generally set forth in Example 1L, the analog [D-4Aph(Cbm)^6]-Acyline is synthesized. After deprotecting the side chain of the 5-position residue, a reaction is carried out with acetic anhydride. The peptide Ac-β-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(Ac)-D-4Aph (carbamoyl)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 99 percent. The peptide is more water soluble than Acyline. MS analysis shows a mass of about 1533.6 Da, which is in agreement with the calculated mass of 1533.7 Da. The peptide is assayed as in Example 1, and at a dosage of 50 micrograms, the peptide exhibits a long duration of suppression of LH levels. It is substantially the same as Acyline over 3 days, and after 96 hours, its suppression is slightly superior to Acyline.

EXAMPLE 1Q

The synthesis as set forth in Example 1P is repeated but reversing the reaction of the deprotected side chains of the two residues in the 5- and 6-positions to create the analog [4-Aph(Cbm)^5]-Acyline. The peptide Ac-β-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(carbamoyl)-D-4Aph(Ac)-Leu-Lys (isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 97 percent. The peptide is more water soluble than Acyline. MS analysis shows a mass of about 1533.6 Da, which is in agreement with the calculated mass of 1533.7 Da. The peptide is assayed in the standard in vivo rat test as in Example 1, and at a dosage of 50 micrograms, it is found to exhibit a potency for the suppression of LH level equal to about values of Acyline over 2 days. Thereafter, the biopotency begins to drop and is not as effective as Acyline.

EXAMPLE 2

The peptide [4Aph(Hor)^5, D-Cit^6]-Antide, an analog of the peptide Cetrorelix having the formula Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(hydroorotyl)-D-Cit-Leu-ILys-Pro-D-Ala-NH_2 is synthesized using the synthesis as generally set forth in Example 1. Instead of coupling N^{α}Boc-D-4Aph in the 6-position, N^{α}Boc-D-Cit is coupled in the 6-position. Alternatively, N^{α}Boc-D-Orn(Fmoc) is coupled in the 6-position, and the chain elongation is temporarily halted after, having obtained the following peptide intermediate: Boc-D-Orn(Fmoc)-Leu-Lys(Ipr,Z)-Pro-D-Ala-NH-[MBHA resin support]. The amino side chain on the Orn residue is then deprotected by removal of the Fmoc protection as in Example 1, and the intermediate is treated with excess t-butyl isocyanate in DMF about 6 hours at room temperature to react with the side chain of the Orn residue. The completion of the synthesis of the decapeptide intermediate is then carried out as in Example 1.

The peptidoresin is then subjected to the standard wash, and cleavage from the resin and deprotection, followed by purification, are carried out as described in Example 1. The peptide Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph (hydroorotyl)-D-Cit-Leu-ILys-Pro-D-Ala-NH_2 (Peptide No. 2) is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 99 percent. LSIMS analysis shows measured mass of 1583.7 Da which is in agreement with the calculated mass of 1583.8 Da for this peptide.

The peptide is more hydrophilic than Cetrorelix and exhibits as long duration of bioactivity as Cetrorelix when tested in vivo for suppression of LH secretion as in Example 1. It has marginally better suppression at 3 days and substantially better at 96 hours.

EXAMPLE 2A

An analog of the peptide Antide, i.e. [4Aph(Hor)^5]-Antide is synthesized using the synthesis as generally set forth in Example 1 of U.S. Pat. No. 5,169,935. After coupling N^{α}Boc-D-Lys(Fmoc) in the 6-position, it is reacted with an excess of nicotinic acid in DMF following removal of deprotection. Then, N^{α}Boc-Aph(Fmoc) is coupled in the 5-position, and the amino side chain on the Aph residue is then deprotected by removal of the Fmoc protection as in Example 1. The intermediate is reacted with L-hydroortic acid in DMF, and the synthesis of the decapeptide intermediate is completed as in Example 1.

Following the standard wash, cleavage from the resin, deprotection and purification are carried out as described in Example 1. The peptide Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(hydroorotyl)-D-Lys(Nic)-Leu-ILys-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is considered to be more hydrophilic than Cetrorelix and to exhibit as long duration of bioactivity as Cetrorelix for suppression of LH secretion.

EXAMPLE 3

The analog [4Aph(D/L-Imz)^5]-Acyline is synthesized using the synthesis as generally set forth in Example 1B.

Instead of reacting 4Aph in the 5-position with hydroorotic acid, once the side chain is deprotected by removal of the Fmoc protection, the intermediate is treated with an excess of D/L-2-Imidazolidone-4-carboxylic acid and about 90 mg of HOBt in DMF solution for about 6 hours at room temperature to react with the side chain of the 4Aph residue. The completion of the synthesis of the decapeptide intermediate is then carried out as in Example 1.

The peptidoresin is then subjected to the standard wash, deprotection and cleavage from the resin, followed by purification, are carried out as described in Example 1. The peptide Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(D/L-2-imidazolidone-4-carbonyl)-D-4Aph(Ac)-Leu-ILys-Pro-D-Ala-NH_2 (Peptide No. 3) is obtained in the RP-HPLC purification. It is judged to be homogeneous mixture of two compounds, without other impurities. LSIMS analysis shows a measured mass of 1602.7 Da which is in agreement with the calculated mass of 1602.8 Da for this peptide.

Assaying the peptide using the standard in vivo rat test as in Example 1 shows that, at a dosage of 50 micrograms, the peptide exhibits long duration of suppression of LH secretion. It has marginally better suppression at 3 days and at 96 hours than Acyline.

EXAMPLE 3A

The synthesis of Example 3 is repeated using an excess of L-2-imidazolidone-4-carboxylic acid. The resultant peptide is judged to be homogeneous and its purity is estimated to be about 99 percent. LSIMS analysis shows a measured mass of 1602.5 Da, which is in agreement with the calculated mass of 1602.8 Da for this peptide. The peptide is more water soluble than Acyline.

Assaying is carried out as in Example 1, and at a dosage of 50 micrograms, the peptide exhibits long duration of suppression of LH secretion, being about the same as Acyline over a period of 96 hours.

EXAMPLE 3B

A synthesis generally the same as that of Example 3A is carried out. The peptide [4Aph(Imz)^5, D-4Amf(Cbm)^6]-Acyline is synthesized using a combined synthesis as generally taught in Examples 1P and 3A, but coupling N^{α}Boc-D-4Amf(Fmoc) in the 6-position. The peptide Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(L-Imz)-D-4Amf(carbamoyl)-Leu-ILys-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 98 percent. LSIMS analysis shows a measured mass of 1617.6 Da which is in agreement with the calculated mass of 1617.8 Da for this peptide. The peptide is assayed as in Example 1, and at a dosage of 50 micrograms, it exhibits a long duration of suppression of LH secretion. It is substantially the same as Acyline over 3 days and has a somewhat superior suppression at 96 hours.

EXAMPLE 4

The peptide [4Aph(Hor)^5, D-4Amf(MeCbm)^6]-Antide, having the formula Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(hydroorotyl)-D-4Amf(MeCbm)-Leu-Ilys-Pro-D-Ala-NH_2 is synthesized using the synthesis as generally set forth in Example 1A. Instead of reacting D-4Amf in the 6-position with excess t-butyl isocyanate in DMF or DCM, it is caused to react with methyl isocyanate. The completion of the synthesis of the decapeptide intermediate is then carried out as in Example 1A.

The peptidoresin is then subjected to the standard wash, and cleavage from the resin and deprotection, followed by purification, are carried out as described in Example 1. The peptide Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(hydroorotyl)-D-4Amf(MeCbm)-Leu-ILys-Pro-D-Ala-NH_2 (Peptide No. 4) is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 99 percent. LSIMS analysis shows measured mass of 1659.8 Da which is in agreement with the calculated mass of 1659.8 Da for this peptide.

Assaying the peptide using the standard in vivo rat test shows that, at a dosage of 50 micrograms, Peptide No. 4 exhibits better suppression of LH secretion than Acyline and is considered to exhibit very long-acting duration of bioactivity.

EXAMPLE 4A

The synthesis of Example 4 is repeated substituting acetic anhydride for methyl isocyanate to create the peptide [4Aph(Hor)^5, D-4Amf(Ac)^6]-Antide. The peptide Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(hydroorotyl)-D-4Amf(Acm)-Leu-ILys-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 99 percent. LSIMS analysis shows a measured mass of 1644.5 Da which is in agreement with the calculated mass of 1644.8 Da for this peptide.

The peptide is assayed as in Example 1 at a dosage of 50 micrograms, and it exhibits long-acting duration of bioactivity. It shows suppression of LH secretion equal to Acyline for 3 days and at 96 hours is slightly superior to Acyline.

EXAMPLE 5

The peptide [4Aph(D-Hor)^5, D-4Amf(Cbm)^6]-Antide, one which has the formula Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(D-hydroorotyl)-D-4Amf(Cbm)-Leu-ILys-Pro-D-Ala-NH_2 is synthesized using the synthesis as generally set forth in Example 1A. Instead of reacting 4Aph in the 5-position with L-hydroorotic acid, the side chain is reacted with D-hydroorotic acid. The completion of the synthesis of the decapeptide intermediate is then carried out as in Example 1A.

The peptidoresin is then subjected to the standard wash, and cleavage from the resin and deprotection, followed by purification, are carried out as described in Example 1. The peptide Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(D-hydroorotyl)-D-4Amf(Cbm)-Leu-ILys-Pro-D-Ala-NH_2 (Peptide No. 5) is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 98 percent. LSIMS analysis shows measured mass of 1645.8 Da which is in agreement with the calculated mass of 1645.8 Da for this peptide.

Assaying the peptide using the standard in vivo rat test shows that, at a dosage of 50 micrograms, the peptide exhibits a duration of bioactivity in the suppression of LH secretion over 2 days about as long as Acyline and continues to effect some lesser degree of suppression at 72 and 96 hours.

EXAMPLE 5A

The synthesis of Example 5 is repeated except that, instead of reacting the deprotected side chain of 4Amf with t-butyl isocyanate, it is reacted with acetic anhydride. The peptide Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(D-hydroorotyl)-D-4Amf(Ac)-Leu-ILys-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 99 percent. LSIMS analysis shows a measured mass of 1644.7 Da which is in agreement with the calculated mass of 1644.8 Da for this peptide.

The peptide is assayed as in Example 1, and at a dosage of 50 micrograms, the peptide exhibits a suppression of LH secretion substantially the same as Acyline over 3 days; at 96 hours, it exhibits a suppression of LH somewhat superior to Acyline.

EXAMPLE 6

The synthesis as generally set forth in Example 1F is repeated with the exception that N^{α}Boc-D-4Amf(Fmoc) is used for the 6-position residue instead of N^{α}Boc-D-4Aph (Fmoc) to form the decapeptide [4Amf(Hor)^5, D-4Amf (Ac)]-Antide. The peptide Ac-β-D-2Nal-D-4Cpa-D-3Pal-Ser-4Amf(hydroorotyl)-D-4Amf(acetyl)-Leu-Lys (isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 99 percent. MS analysis shows a mass of 1658.7 Da, which is in agreement with the calculated mass of 1658.8 Da.

The peptide is assayed as in Example 1, and at a dosage of 50 micrograms, the peptide is found to have long-acting duration in the suppression of LH secretion. It is about the same as Acyline over the first two days and exhibits a biopotency nearly equal to that of Acyline over days 3 and 4.

EXAMPLE 6A

The synthesis of Example 6 is repeated, except that instead of reacting the deprotected side chain of D-4Amf with acetic anhydride, it is reacted with t-butyl isocyanate as in Example 1 to form the peptide [4Amf (Hor)^5, D-4Amf (Cbm)^6]-Antide. The decapeptide Ac-β-D-2Nal-D-4Cpa-D-3Pal-Ser-4Amf(hydroorotyl)-D-4Aph(carbamoyl)-Leu-Lys (isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be about 99 percent. MS analysis shows a mass of 1659.6 Da, which is in agreement with the calculated mass of 1659.8 Da.

The peptide is assayed as in Example 1, and at a dosage of 50 micrograms, it is as active as Acyline in the suppression of LH secretion after 1 day and nearly as active after 2 days. It is somewhat less active after 3 days but exhibits about the same activity as Acyline after 4 days.

EXAMPLE 6B

The synthesis of Example 6A is repeated, except that the reaction is carried out with methyl isocyanate instead of t-butyl isocyanate to create the peptide [4Amf(Hor)^5, D-4Amf(MeCbm)^6]-Antide. The peptide Ac-β-D-2Nal-D-4Cpa-D-3Pal-Ser-4Amf(hydroorotyl)-D-4Aph (methylcarbamoyl)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be about 99 percent. MS analysis shows a mass of 1673.6 Da, which is in agreement with the calculated mass of 1673.8 Da.

The peptide is tested in the assay as set forth in Example 1, and at a dosage of 50 micrograms, the peptide is as active as Acyline in the suppression of LH secretion after 1 day and about as active after 2 days. At 3 and 4 days, it continues to effect a significantly lesser degree of suppression of LH secretion than Acyline.

EXAMPLE 6C

The synthesis of Example 6 is repeated, substituting D-hydroorotic acid for L-hydroorotic acid to form the peptide [4Aph(D-Hor)^5, D-4Amf(Ac)^6]-Antide. The peptide Ac-β-D-2Nal-D-4Cpa-D-3Pal-Ser-4Amf(D-hydroorotyl)-D-4Aph(acetyl)-Leu-Lys(isopropyl)-Pro-D-Ala-NH_2 is obtained in the RP-HPLC purification. It is judged to be homogeneous, and its purity is estimated to be greater than 99 percent. MS analysis shows a mass of 1658.7 Da, which is in agreement with the calculated mass of 1658.8 Da.

The peptide is assayed as in Example 1, and at a dosage of 50 micrograms, it is substantially as effective Las Acyline for days 1 and 2. At day 3, it is substantially less effective than Acyline and continues to significantly drop in biopotency thereafter.

EXAMPLE 7

Using the procedures as generally set forth in Examples 1 to 5, the following GnRH antagonist peptides are also prepared:

[Acr-D-2Nal^1,4FD-Phe^2,4Aph(Hor^5)]-Acyline

[Bz-D-2Nal^1,4NO_2D-Phe^2,4Aph(Hor)^5,D-4Aph(Hor)^6]-Antide

[For-D-2Nal^1,4OCH_3D-Phe^2,4Amf(Hor)^5,D-4Aph(D-Hor)^6]-Antide

[Acr-D-2Nal^1,4BrD-Phe^2,4Aph(Imz)^5,D-4Aph(Imz)^6]-Antide

[Pn-D-2Nal^1,4CH_3D-Phe^2,4Aph(MeCbm)^5,D-4Aph(D-Hor)^6]-Antide

[Bt-D-2Nal^1,3,4Cl_2D-Phe^2,4Aph(Cbm)^5,D-4Aph(Hor)^6]-Antide

[Vl-D-2Nal^1,4NO_2D-Phe^2,4Aph(Hor)^5,D-4Aph(Cbm)^6]-Antide

[Vac-D-2Nal^1,C^{α}Me4ClD-Phe^2,4Aph(Hor)^5,Gly^{10}]-Acyline

[Acr-D-2Nal^1,4Aph(Hor)^5,Arg(Et_2)^8,D-Agl(Me)^{10}]-Acyline

[MeCbm-D-2Nal^1,4Aph(Cbm)^5,Arg^8,Agl(Me)^{10}]-Acyline

[Cbm-D-2Nal^1,4Amf(MeCbm)^5,Ala^{10}]-Acyline

[EtCbm-D-2Nal^1,4Amf(iprCbm)^5,Pro^9$NHCH_2CH_3$]-Acyline

[Acr-D-2Nal^1,4Aph(Imz)^5,D-4Amf(Cbm)^6,Arg^8]-Antide

[Cbm-D-2Nal^1,4Aph(MeCbm)^5,D-4Amf(D-Hor)^6,Arg(Et_2)^8]-Antide

[4Ahp(Hor)^5, D-4Ahp(Imz)^6,D-Agl^{10}]-Antide

[Ac-D-1Nal^1,4Amf(Hor)^5,D-4Amf(D-Hor)^6,Arg^8]-Antide

[PrCbm-D-2Nal^1,4Amf(Imz)^5,D-4Ahp(EtCbm)^6, Pro^9$NHCH_2CH_3$]-Antide

[4Amf(Hor)^5,D-NicLys^6,AzaGly^{10}]-Antide

[4Amf(Hor)^5,D-Cit^6,Har(Et_2)^8]-Antide

[4Aph(Hor)^5,D-Lys(Nic)^6,D-Agl^{10}]-Antide

[4Aph(Hor)^5,D-Hci^6,Agl(Me)^{10}]-Antide

[4Aph(Hor)^5,D-3Pal^6,Har^8,Agl^{10}]-Antide

[4Aph(Hor)^5,D-4Aph(For)^6,D-Agl(Me)^{10}]-Antide

[4Aph(Hor)^5,D-4Aph(atz)^6,Har(Et_2)^8]-Antide

[4Aph(Hor)^5,D-4Aph(iprCbm)^6,D-Agl^{10}]-Antide

[For-D-1Nal^1,4Amf(Hor)^5,D-4Amf(atz)^6,Gly^{10}]-Antide

[4Aph(D-Hor)^5,D-4Aph(Cbm)^6,Ala^{10}]-Antide

These peptides are biopotent in inhibiting the secretion of LH.

The foregoing compounds which were tested were shown to exhibit biological potency in the suppression of LH to an extent at least generally comparable to the corresponding GnRH antagonist peptide known as Antide, of which they are considered to be analogs. As a result of extensive testing in this area for over a decade, biopotency determined in this widely accepted test measuring the suppression of LH has been accepted as evidence as to such compounds' ability to suppress gonadotropin secretion and thus to exhibit useful antigonadal, anti-ovulatory effects. Based upon superior solubility, resistance to in vivo gelling, long duration of bioactivity and other properties, these compounds are considered to be generally useful as antigonadal agents to suppress the secretion of gonadotropins and inhibit the release of steroids by the gonads, e.g. as anti-ovulatory agents.

The compounds of the invention are often administered in the form of pharmaceutically acceptable, nontoxic salts, such as acid addition salts, or of metal complexes, e.g., with zinc, barium, calcium, magnesium, aluminum or the like (which are considered as addition salts for purposes of this application), or of combinations of the two. Illustrative of such acid addition salts are hydrochloride, hydrobromide,

sulphate, phosphate, nitrate, oxalate, fumarate, gluconate, tannate, pamoate, maleate, acetate, citrate, benzoate, succinate, alginate, malate, ascorbate, tartrate and the like; acetate and pamoate, the salt of pamoic acid, may be preferred. If the active ingredient is to be administered in tablet form, the tablet may contain a pharmaceutically-acceptable, nontoxic diluent which includes a binder, such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid; and a lubricant, such as magnesium stearate. If administration in liquid form is desired, sweetening and/or flavoring may be used as part of the pharmaceutically-acceptable diluent, and intravenous administration in isotonic saline, phosphate buffer solutions or the like may be effected.

The pharmaceutical compositions will usually contain an effective amount of the peptide in conjunction with a conventional, pharmaceutically-acceptable carrier or diluent. Usually, the dosage will be from about 10 micrograms to about 2.5 milligrams of the peptide per kilogram of the body weight of the host when given intravenously. The nature of these compounds may permit effective oral administration; however, oral dosages might be higher. Overall, treatment of subjects with these peptides is generally carried out in the same manner as the clinical treatment using other antagonists of GnRH, using a suitable carrier in which the compound is soluble and administering a dosage sufficient to suppress LH and FSH levels in the patient.

It may also be desirable to deliver the GnRH analog over prolonged periods of time, for example, for periods of one week to one year from a single administration, and slow release, depot or implant dosage forms may be utilized. For example, a suitable, slow-release depot formulation for injection may contain the GnRH antagonist or a salt thereof dispersed or encapsulated in a slow degrading, non-toxic or non-antigenic polymer such as a polylactic acid/polyglycolic acid polymer, for example, as described in U.S. Pat. No. 3,773,919. It is also known to administer such slow-release dosage formulations by a poultice that may be applied within the mouth. These compounds may also be formulated into silastic implants.

These compounds can be administered to mammals intravenously, subcutaneously, intramuscularly, orally, percutaneously, e.g. intranasally or intravaginally to achieve fertility inhibition and/or control and also in applications calling for reversible suppression of gonadal activity, such as for the management of precocious puberty or during radiation- or chemotherapy. They are also useful for treatment of steroid-dependent tumors. Effective dosages will vary with the form of administration and the particular species of mammal being treated. An example of one typical dosage form is a bacteriostatic water solution at a pH of about 6 containing the peptide which solution is administered parenterally to provide a dose in the range of about 0.1 to 2.5 mg/kg of body weight per day. These compounds are considered to be well-tolerated in vivo and to resist gelling; accordingly, they are considered to be particularly well-suited for administration by subcutaneous injection in a bacteriostatic water solution at appropriate concentrations, above about 0.75 mg/ml and even above about 1.0 mg/ml, without danger of gelling at the point of injection.

These GnRH antagonist peptides are also useful diagnostically, both in vivo and in vitro. These peptides can be injected in vivo followed by assaying the bloodstream of a patient to determine the extent of decrease of hormonal secretion, e.g. LH secretion. In vitro assays can be carried out to determine whether certain tumor cells are sensitive to GnRH. In such assays, tumor cell cultures are treated with the GnRH antagonist peptides and then monitored for hormonal secretions and cell proliferation.

Although the invention has been described with regard to its preferred embodiments, it should be understood that changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention which is set forth in the claims which are appended hereto. In the synthesis, an isocyanate can be reacted with the amino side chain prior to coupling the α-amino protected amino acid into the peptide chain rather than modifying it while a part of the chain. For example, other substitutions known in the art which do not significantly detract from the effectiveness of the peptides may be employed in the peptides of the invention. Whereas the N-terminus may be left unsubstituted or other equivalent acylating groups can be used, either acetyl or substituted or unsubstituted carbamoyl is preferred. Other substituted D-Phe, such as (4F)D-Phe, can be used in the 2-position. Instead of Aph(Ac), the aminoPhe group can be treated with alternative acylating agents as disclosed in U.S. Pat. No. 5,506,207, such as formic acid, β-Ala(atz) and gamma-aminobutyric acid(atz), which likewise result in GnRH antagonists that exhibit long-acting duration; thus, the resulting residues are considered equivalents of D- and L-4Aph(Ac). Both Lys(Bu) and Lys(Et_2) are considered to be equivalents of lLys; however, lLys is most preferred. Other hydrophobic amino acid residues can also be employed in the 1-position and in the 6-position (as mentioned hereinbefore), preferably in D-isomer form, and are considered equivalents of those specified. Moreover, the antagonists can be administered in the form of their pharmaceutically or veterinarially acceptable, nontoxic salts, as indicated hereinbefore, which are considered equivalents.

The disclosures of all U.S. patents hereinbefore mentioned are incorporated herein by reference. Particular features of the invention are emphasized in the claims which follow.

What is claimed is:

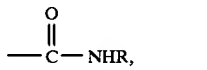
1. A GnRH antagonist peptide having the formula:

X-D-2Nal-(A)D-Phe-D-3Pal-Ser-Xaa_5-Xaa_6-Leu-Xaa_8-Pro-Xaa_{10}

or pharmaceutically acceptable salt thereof wherein:

X is an acyl group having not more than 7 carbon atoms or Q,

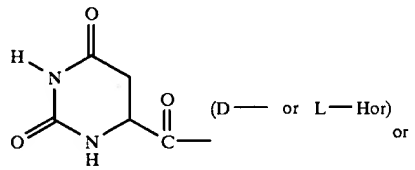
with Q being

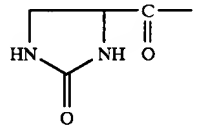


and with R being H or lower alkyl;

A is 4Cl, 4F, 4Br, $4NO_2$, $4CH_3$, $4OCH_3$, $3,4Cl_2$ or C^{α}Me4Cl;

Xaa_5 is 4Aph(Q_1) or 4Amf(Q_1) with Q_1 being





Xaa_6 is D-4Aph(Q_2), D-4Amf(Q_2), D-Lys(Nic), D-Cit, D-Hci or D-3Pal, with Q_2 being For, Ac, 3-amino-1,2,4-triazole, Q or Q_1;

Xaa_8 is Lys(ipr), Arg, Har, Arg(Et_2) or Har(Et_2); and

Xaa_{10} is D-Ala-NH_2, $NHCH_2CH_3$, Gly-NH_2, Ala-NH_2, AzaGly-NH_2, Agl-NH_2, D-Agl-NH_2, Agl(Me)-NH_2 or D-Agl(Me)-NH_2.

2. A GnRH antagonist according to claim **1** wherein Q_1 is L-Hor or D-Hor.

3. A GnRH antagonist according to claim **2** wherein Q_2 is Q and R is H or methyl.

4. A GnRH antagonist according to claim **2** wherein Xaa_6 is D-4Aph(D-Hor).

5. A GnRH antagonist according to claim **2** wherein X is Ac.

6. A GnRH antagonist according to claim **2** wherein Xaa_8 is Lys(ipr).

7. A GnRH antagonist according to claim **2** wherein Xaa_{10} is D-Ala-NH_2.

8. A GnRH antagonist according to claim **2** wherein X is —$CONHCH_3$.

9. A GnRH antagonist according to claim **1** wherein Xaa_5 is 4Aph(L- or D-Hor) and Xaa_6 is D-4Aph(Ac), D-4Aph (atz), or D-3Pal.

10. A GnRH antagonist according to claim **1** wherein Xaa_5 is 4Aph(L- or D-Hor) and Q_2 is Q and R is H or methyl.

11. A GnRH antagonist according to claim **1** wherein Xaa_5 is 4Aph(L- or D-Hor) and Xaa_6 is D-Cit or D-Hci.

12. A GnRH antagonist peptide according to claim **1** having the formula:

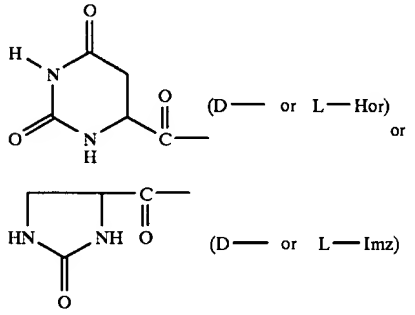
X-D-2Nal-(A)D-Phe-D-3Pal-Ser-Xaa_5-Xaa_6-Leu-Lys (ipr)-Pro-Xaa_{10}

wherein:

X is For, Ac, Acr, Pn, Bt, Vl, Vac, Bz or Q, with Q being defined as in claim **1**;

A is 4Cl or 4F;

Xaa_5 is 4Aph(Q_1) or 4Amf(Q_1) with Q_1 being a D-isomer, an L-isomer, or a D/L-isomer mixture of either



Xaa_6 is D-4Aph(Q_2), D-4Amf(Q_2), D-Cit, D-Lys(Nic) or D-3Pal, with Q_2 being For, Ac, Q or Q_1; and

Xaa_{10} is D-Ala-NH_2, $NHCH_2CH_3$ or Gly-NH_2.

13. A GnRH antagonist according to claim **12** wherein Q_1 is L- or D-Hor and Xaa_6 is D-4Amf(Q), with R being H or methyl.

14. A GnRH antagonist peptide according to claim **12** wherein X is Ac or Q; R is H or methyl; Xaa_6 is D-4Aph (Q_2), D-4Amf(Q_2) or D-3Pal, with Q_2 being Ac, Q or Q_1; and Xaa_{10} is D-Ala-NH_2.

15. A GnRH antagonist according to claim **1** having the formula: Ac-D-2Nal-D-4ClPhe-D-3Pal-Ser-4Aph(L-Hor)-Xaa_6-Leu-Lys(ipr)-Pro-D-Ala-NH_2, wherein Xaa_6 is D-4Aph(Ac), D-3Pal, D-4Aph(carbamoyl), D-4Amf (carbamoyl), D-4Amf(methylcarbamoyl) or D-4Aph(D-Hor).

16. The GnRH antagonist according to claim **15** wherein Xaa_6 is D-4Aph(carbamoyl).

17. The GnRH antagonist according to claim **15** wherein Xaa_6 is D-4Amf(carbamoyl).

18. A GnRH antagonist according to claim **1** wherein Xaa_5 is 4Aph(L-Hor) and Xaa_6 is D-Aph(Q) or D-Amf(Q) with R being H or methyl.

19. A pharmaceutical composition for inhibiting the secretion of gonadotropins in mammals comprising, as an active ingredient, an effective amount of a GnRH antagonist according to claim **1** in association with a nontoxic diluent.

20. A method for inhibiting the secretion of gonadotropins in mammals comprising administering an amount of a pharmaceutical composition according to claim **19** which effects a substantial decrease in LH and FSH levels.

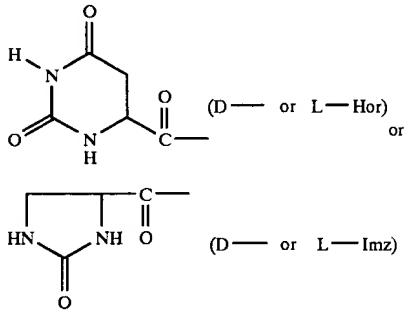
21. An intermediate for making a GnRH antagonist peptide having the formula: X^1-D-2Nal-(A)D-Phe-D-3Pal-Ser (X^2)-Xaa_5-Xaa_6-Leu-Lys(ipr) (X^4)-Pro-X^5 wherein:

X^1 is an α-amino-protecting group;

A is 4Cl or 4F;

X^2 is an hydroxyl-protecting group;

Xaa_5 is 4Aph(Q_1) or 4Amf(Q_1) with Q_1 being a D-isomer, an L-isomer or a D/L-isomer mixture of either



Xaa_6 is D-4Aph(Q_2), D-4Amf(Q_2) or D-3Pal, with Q_2 being Ac, Q_1, carbamoyl or methylcarbamoyl;

X^4 is an acid-labile amino-protecting group; and

X^5 is D-Ala-, Gly-, Ala-, Agl-, D-Agl-, Agl(Me)-, or D-Agl(Me)-resin support; or N(Et)-resin support; an amide of D-Ala, Gly or Ala; ethylamide; or AzaGly-NH_2.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
Certificate

Patent No. 5,925,730 Patented: July 20, 1999

On petition requesting issuance of a certificate for correction of inventorship pursuant to 35 U.S.C. 256, it has been found that the above identified patent, through error and without any deceptive intent, improperly sets forth the inventorship.

Accordingly, it is hereby certified that the correct inventorship of this patent is: Graeme Semple, Gothenburg, Sweden; Guangcheng Jiang, San Diego, CA; and Jean E. F. Rivier, La Jolla, CA.

Signed and Sealed this Fourteenth Day of September 2004.

SREENI PADMANABHAN
Supervisory Patent Examiner
Art Unit 1617

ATTACHMENT E

In re U.S. Patent No. 5,925,730
Issued: July 20, 1999
To: Graeme Semple et al.
Assignee: Ferring BV
For: GNRH ANTAGONISTS
Application for Patent Term Extension
Customer No. 22852

Attachment E

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Customer No 000000

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DATE PRINTED
02/04/2009

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SUITE 1600
CHICAGO IL 60603-3406

MAINTENANCE FEE STATEMENT

According to the records of the U.S.Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed below. The "PYMT DATE" column indicates the payment date (i.e., the date the payment was filed).

The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O.Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
5,925,730	**$2,300.00**	**$0.00**	**12/29/06**	**08/837,042**	**07/20/99**	**04/11/97**	**08**	**NO**	**57714**

ATTACHMENT F

In re U.S. Patent No. 5,925,730
Issued: July 20, 1999
To: Graeme Semple et al.
Assignee: Ferring BV
For: GNRH ANTAGONISTS
Application for Patent Term Extension
Customer No. 22852

BRIEF DESCRIPTION OF ACTIVITIES DURING REGULATORY REVIEW PERIOD OF DEGARELIX

Date	Description of Correspondence
07/09/01	Original IND Submission
07/13/01	FROM FDA - Acknowledgement of Receipt of IND
07/31/01	FROM FDA – Comments Regarding Clinical Study
08/02/01	Protocol Amendment
08/06/01	Protocol Amendment
08/07/01	FROM FDA – IND CMC Comments
08/15/01	FROM FDA – IND Placed on Partial Clinical Hold
09/13/01	FROM FDA – Minutes of 08.06.01 Teleconference
09/18/01	Clinical Study Protocol
02/25/02	Amendment – Clinical Studies
03/12/02	Clinical Study Protocol - Amendment
03/27/02	Protocol Amendment
04/05/02	Letter Concerning Clinical Protocols
04/09/02	Protocol Amendment
04/10/02	FROM FDA - Protocols May Proceed
04/26/02	Protocol Amendment
04/29/02	FROM FDA – Meeting Minutes From 04.04.02 Teleconference
05/16/02	Protocol Amendment
05/16/02	Protocol Amendment
05/30/02	Request for Special Protocol Assessment for Carcinogenicity Protocols
06/28/02	FROM FDA - Response to Carcinogenicity Special Protocol Assessment Request
07/18/02	Protocol Amendment
08/09/02	Toxicology Studies
08/09/02	Fax to FDA – Re: Follow-up to 08.09.02 Teleconference
08/14/02	FROM FDA – Protocol Amendment
09/13/02	Protocol Amendment
11/07/02	Amendment – Clinical Data
01/13/03	Protocol Amendment
04/14/03	Protocol Amendment
05/02/03	Protocol Amendment
05/22/03	FROM FDA – Re: Clinical Protocols
07/18/03	Protocol Amendment
08/28/03	Request for Meeting
10/09/03	Meeting – Information Package
10/15/03	Protocol Amendment – New Protocol With Revised Investigator's Brochure
11/24/03	FROM FDA - Confirmation of Meeting Rescheduled for 01.26.2004
01/19/04	Protocol Amendment
01/26/04	FROM FDA - 01.26.2004 Meeting Minutes
02/11/04	Protocol Amendment
03/18/04	Non-Clinical Protocol
03/18/04	FROM FDA - 02.15.2004 Meeting Minutes
04/14/04	FROM FDA - Comments on Clinical Study
04/22/04	FROM FDA - Toxicology Study
05/14/04	Protocol Pharm-Tox Study
06/03/04	FROM FDA - Carcinogenicity Study

06/21/04	Protocol Pharm-Tox Study
07/13/04	FROM FDA - Carcinogenicity Study
07/22/04	Protocol Pharm-Tox Study
07/29/04	Request for Type B Meeting - CMC
08/06/04	Protocol Amendment
08/06/04	Request for Type B CMC Reschedule
08/17/04	FROM FDA – CMC Meeting Scheduled for 10.26.2004
08/19/04	FROM FDA - Carcinogenicity Study in Mice
09/23/04	Information Package for EoP2 CMC Meeting
10/07/04	Protocol Amendment
10/20/04	Responses to Questions For EoP2 Meeting Scheduled 10.26.2004
10/25/04	Information Amendment – Pharmacology and Toxicology
10/29/04	FROM FDA - Comments and Responses to Minutes of 02.14.2004 Meeting
11/02/04	FROM FDA – Questions and Responses to Information Package Sent 09.23.2004
12/13/04	FROM FDA - Submission of Carcinogenicity Data
01/07/05	Request for Type B EoP2 Meeting
01/10/05	Transfer of IND Sponsor Obligations to a CRO
01/14/05	Protocol Amendment
01/21/05	FROM FDA – Meeting Scheduled for EoP2
01/28/05	FROM FDA - Fax – Questions for Sponsor for 03.14.2005 Meeting
02/01/05	New Protocol
02/09/05	EOP2A Meeting Information Package
02/18/05	Electronic Files for EOP2A Meeting
02/22/05	FROM FDA - Teleconference Scheduled 02.24.2005
03/08/05	Response to FDA Questions
03/25/05	Protocol Amendment
04/05/05	Sponsor's Minutes of 03.14.2005 Meeting
04/12/05	FROM FDA – Meeting Minutes of 03.14.2005 Meeting
07/01/05	Transfer of IND Sponsor Obligations to a CRO
07/01/05	Transfer of IND Sponsor Obligations to a CRO
07/07/05	Request for Type B – End of Phase 2 Meeting
08/04/05	FROM FDA – EOP2 Meeting
09/07/05	Information Package for 09.30.2005 EoP2 Meeting
09/28/05	FROM FDA – 09.30.2005 Meeting
10/13/05	FROM FDA – 09.30.2005 Meeting
12/06/05	Request for Special Protocol Assessment
01/19/06	FROM FDA – Comments on Meeting
02/17/06	FROM FDA - Response to Special Protocol Assessment
02/17/06	FROM FDA – Minutes of EOP2 Meeting
02/23/06	Protocol Amendment
02/28/06	Protocol Amendment
02/28/06	Case Report Form for Clinical Study
03/30/06	FROM FDA - Comments on 02.28.06 Submission
05/11/06	New Protocol
06/26/06	Transfer of Obligations to a CRO
08/02/06	New Clinical Protocol
11/03/06	Request for Type B - End of Phase 2 Meeting
11/29/06	FROM FDA – EoP2 Meeting Information
12/08/06	Information Package for EoP2 Meeting

12/19/06	Protocol Amendment – Clinical Study
01/10/07	FROM FDA - Pre-Meeting Comments – EoP2 Meeting
01/10/07	FROM FDA - Pre-Meeting Comments
01/12/07	New Clinical Protocol
01/15/07	FROM FDA – Minutes of 01.10.2007 Teleconference
03/20/07	Clinical Protocol
04/16/07	New Clinical Protocol
05/24/07	Statistical Analysis Plan
07/19/07	Request for Type B Pre-NDA Meeting
08/10/07	Amendment to Statistical Analysis Plan
08/21/07	FROM FDA – Pre-NDA Meeting
08/22/07	Transfer of IND Sponsor Obligations to a CRO
08/22/07	Transfer of IND Sponsor Obligations to a CRO
10/12/07	Amendment to Statistical Analysis Plan
10/12/07	Information Package for Pre-NDA Meeting Scheduled 10.17.2007
10/25/07	FROM FDA – Minutes of 10.17.2007 Meeting
12/20/07	Protocol Amendment
12/21/07	Request for Special Protocol Assessment
02/07/08	FROM FDA – Clinical Studies
02/28/08	NDA received by FDA
03/03/08	Protocol Amendment – Clinical Study
03/28/08	Amendment for Clinical Protocol
06/06/08	Amendment to Clinical Protocol
10/17/08	New Clinical Protocol – Request for Special Protocol Assessment
10/17/08	Transfer of IND Sponsor Obligations to a CRO
12/03/08	FROM FDA - Response to Questions on Clinical Protocol
12/24/08	FROM FDA – NDA Approval Letter

ATTACHMENT G

In re U.S. Patent No. 5,925,730
Issued: July 20, 1999
To: Graeme Semple et al.
Assignee: Ferring BV
For: GNRH ANTAGONISTS
Application for Patent Term Extension
Customer No. 22852

PATENT
Atty. Docket No.: 10192.0022

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent No. 5,925,730)
)
Issued: July 20, 1999)
)
To: Graeme Semple, Guangcheng Jiang,)
Jean E. F. Rivier)
)
Assignee: Ferring BV)
)
For: GNRH ANTAGONISTS)

ATTN: MAIL STOP HATCH-WAXMAN PTE **Attachment G**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

CERTIFICATION

I, CHARLES E. VAN HORN, do hereby certify that this accompanying application for extension of the term of U.S. Patent No. 5,925,730 under 35 U.S.C. § 156 including its attachments and supporting papers is being submitted as one original and two (2) copies thereof.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Date: February 12, 2009

By: Charles E Van Horn
Charles E. Van Horn
Reg. No. 40,266